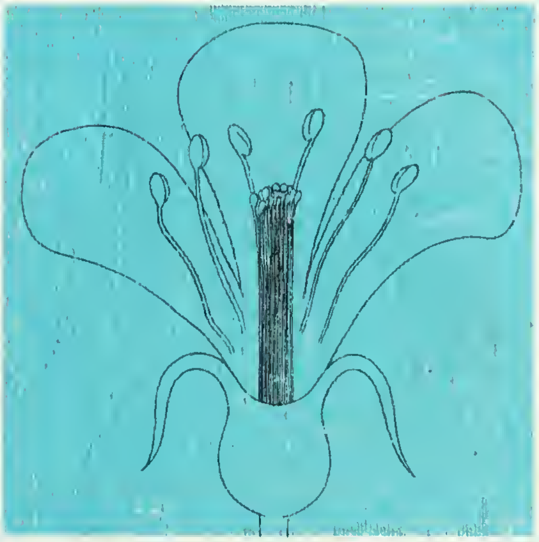
BIOLOGY

Teachers'guide II *Life and living processes*



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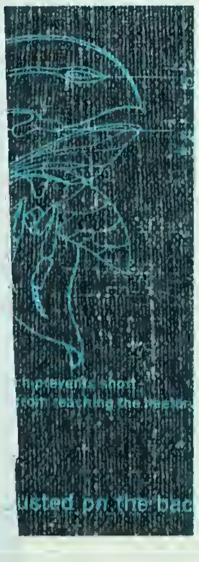

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Nuffield Biology Teachers' Guide II **Life and living processes**

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Nuffield Biology Teachers' Guide II

Life and living processes

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Foreword

This volume is one of the first to be produced by the Nuffield Science Teaching Project, whose work began early in 1962. At that time many individual schoolteachers and a number of organizations in Britain (among whom the Scottish Education Department and the Association for Science Education, as it now is, were conspicuous) had drawn attention to the need for a renewal of the science curriculum and for a wider study of imaginative ways of teaching scientific subjects. The trustees of the Nuffield Foundation considered that there were great opportunities here. They therefore set up a science teaching project and allocated large resources to its work.

The first problems to be tackled were concerned with the teaching of O-Level physics, chemistry, and biology in secondary schools. The programme has since been extended to the teaching of science in sixth forms, in primary schools, and in secondary school classes which are not studying for O-Level examinations. In all these programmes the principal aim is to develop materials that will help teachers to present science in a lively, exciting, and intelligible way. Since the work has been done by teachers, this volume and its companions belong to the teaching profession as a whole.

The production of the materials would not have been possible without the wholehearted and unstinting collaboration of the team members (mostly teachers on secondment from schools); the consultative committees who helped to give the work direction and purpose; the teachers in the 170 schools who participated in the trials of these and other materials; the headmasters, local authorities, and boards of governors who agreed that their schools should accept extra burdens in order to further the work of the project; and the many other people and organizations that have contributed good advice, practical assistance, or generous gifts of material and money.

To the extent that this initiative in curriculum development is already the common property of the science teaching profession, it is important that the current volumes should be thought of as contributions to a continuing process. The revision and renewal which will be necessary in the future, will be greatly helped by the interest and the comments of those who use the full Nuffield programme and of those who follow only some of its suggestions. By their interest in the project, the trustees of the Nuffield Foundation have sought to demonstrate that the continuing renewal of the curriculum – in all subjects – should be a major educational objective.

Brian Young
Director of the Nuffield Foundation

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Preface

Teaching methods

Teaching is so much a personal art that it would be wrong to suggest any single, rigid approach. At the same time we must remember that a course very largely succeeds or fails because of the way it is taught. *How* we teach is certainly as important as *what* we teach.

This course incorporates a selection of those teaching methods that appear to us to be most suitable for attaining its aims. Although it is written as a coherent whole, many teachers (initially, at least), may feel unable to adopt it in its entirety. They may lack the proper facilities, time, or experience. Moreover, they may wish to adapt it to suit their own particular circumstances.

Such considerations need not stand in the way of change. We believe, however, that where a reduced course is planned it should have the same broad structure as the text. The aims of the project will not be achieved without proper attention to its main themes and principles. For example, the later years of the course include studies in population problems, genetics, and physiology. But the special circumstances of a school may well lead to a particular emphasis being placed on one or more of these aspects of biology.

Where time is limited, secondhand evidence can, with discretion, be substituted for class practicals. If parallel classes are following the same course it will sometimes be possible for them to exchange findings from different investigations. Moreover, teacher demonstration can, on occasions, be as effective in making a point as a class practical – or even more so. In such ways much can be done to overcome difficulties imposed by lack of time and facilities.

Aims of the course

These aims can be summarized briefly as follows:

a. To develop and encourage an attitude of curiosity and enquiry.

b. To develop a contemporary outlook on the subject.

c. To develop an understanding of man as a living organism and his place in nature:

1. The usefulness and social implications of biology in relation to man's everyday needs, e.g. food and public health.
2. The profound influence of man's activities on other organisms.
3. The way in which a study of biology enables man to interpret observations that he makes in everyday life, e.g. the distribution of plants and animals.

d. To foster a realization of the variety of life and of underlying similarities among living things.

e. To encourage a respect and feeling for all living things.

f. To teach the art of planning scientific investigations, the formulation of questions, and the design of experiments (particularly the use of controls).

g. To develop a critical approach to evidence.

h. To develop the following ideas about biology as part of human endeavour.

1. Biology has been developing over many centuries: there are many unanswered questions about life; our ideas of life may change as new knowledge is obtained.
2. That biological knowledge is the product of scientists working in many different parts of the world. Its pursuit is international.
3. That it is based not only on observation and experimentation but also on questioning, the formulation of hypotheses, testing of hypotheses, and, above all, on communication between people.
4. That developments in chemistry, physics, and mathematics are helping us to make advances in biology.

Preparation and homework

Preparation and homework constitute an integral part of any course of teaching. As far as the Nuffield O-Level Biology Course is concerned, prepared work can fulfil an important role in helping to ensure that the atmosphere of enquiry which we have tried to build up during school hours is not broken every time a pupil leaves the laboratory. Inevitably, a certain amount of time out of school will have to be devoted to the writing-up of practical work (see 'Long-term investigations' below), but we suggest that this is kept to a minimum. Following the advice of the majority of teachers engaged in the preliminary trials of the course, we have not included lists of questions at the end of each chapter. However, in developing each topic in the text we have posed numerous questions to which we have frequently not supplied the answers. These can provide useful material for homework. Further suggestions are made in various sections of the guide. At the end of most chapters in the text we have included a section entitled 'Background reading' – usually an extension of some topic which it has not been possible to develop fully elsewhere. We hope this may also play a part in stimulating additional reading out of school hours. We suggest that such reading could also provide a link between the teaching of Biology and English in a school; essays and discussions might well be based on some of the topics included. Background reading will not be examined in G.C.E. However, we strongly suggest that pupils should look upon it as an integral part of the Course.

Using the text

In writing the text we have borne the following considerations particularly in mind.

a. We have tried to avoid giving the impression that the text is intended to usurp the position of the teacher in relation to the class. On the contrary, we have attempted to suggest in the text and guide a sufficient variety of teaching approaches to enable a teacher to develop those most suited to his own particular aptitudes and personality. Wherever possible however, we have tried to cut down the time spent in class on purely routine activities. For instance, we have attempted to provide a logical introduction to all practical work and adequate instructions for carrying it out in the hope that this will minimize the amount of time spent on preliminary demonstration of equipment and the detailed explanation of techniques. More of the teacher's time can thus be spent in discussion and dealing with the problems of particular pupils. Similarly, the introductory material on a topic can be read by the pupils for preparation in order to save time in class.

b. The text is intended to provide a clear indication of the overall structure of the course. This includes the main divisions of the subject matter; how different topics are related to one another; how the subject matter can be approached in an investigatory manner similar to a scientific enquiry; and how firsthand information (derived from the laboratory) and secondhand information (obtained from the books and film loops) can be related to one another.

c. Throughout the text numerous opportunities are provided for initiating simple investigations and the formulation of hypotheses; questions are posed to which the answers are not, at once, given. In fact, the appropriate results or answers may well be incorporated in subsequent material, but we hope that the method of presentation does not make this too obvious when the pupils are first faced with an open-ended situation.

d. Where investigations cannot be conducted on living organisms, models have sometimes been devised to simulate the structures or functions being investigated. Care is needed to keep the model situations closely related to those obtaining in the living things themselves.

e. No separate laboratory manual has been produced for the course. The text includes details of all laboratory work, our aim being to enable students to carry out individual or group experiments and investigations with the minimum of technical help. Where techniques requiring a number of manipulative processes are involved, the sequence of events has been summarized for easy reference in the form of flow diagrams.

Fitting the course into the school year

While the material contained in each chapter of the text is developed in a logical order, there is no set sequence to the course as a whole. Apart from seasonal considerations which are important for certain sections such as the work on flowering plants, it is intended that teachers should adapt the course freely to suit their own preferences and the facilities available. However, some teachers may feel that, while they are becoming familiar with the new materials, they would prefer to adhere to the existing sequence. For them we suggest that the distribution of time in Year II could be:

Term 1	Chapters 1–4.
Term 2	Chapters 5–7.
Term 3	Chapters 8–10.

Teaching and enquiry

One of the principal aims of the Nuffield Biology Course has been to foster a critical approach to the subject with an emphasis on experimentation and enquiry rather than on mere factual assimilation. How can we foster this 'spirit of enquiry' in our pupils? There is no simple answer to this question for teachers convey an attitude to their subject in a variety of different ways. Moreover, we must bear in mind that with younger pupils it is essential for them to experience a sense of achievement in their practical work. This requirement may well conflict to some extent with the presentation of a genuine experimental situation which inevitably involves some degree of uncertainty. The following suggestions may provide a helpful starting point.

a. We suggest that whenever possible pupils should gain their initial understanding of a basic concept by being faced with a series of problems.

b. The next phase involves the formulation of a hypothesis – the point when curiosity or observation changes to constructive questioning.

c. Where possible, we suggest that deductions from experimental work should always include suggestions for a further stage in the enquiry, thus fostering the idea that one experiment paves the way for the next.

d. We sometimes forget that work done outside school time is just as much an integral part of a course as the teaching itself. Problems and test questions set in a spirit of enquiry can do much to maintain and enhance attitudes acquired in the laboratory.

e. One of the advantages of adopting a more enquiring approach involving a greater use of discussion, is that pupils tend to be forthcoming with ideas – both fruitful and unfruitful. Dealing with good ideas is generally a fairly easy matter; the bad ones sometimes present problems, particularly that of not discouraging the pupils who ask them. Adopting the attitude that every idea is a good one just because it is an idea seems to us to have much to commend it at school level.

The value of discussion

The value of discussion in conveying the spirit and methods of scientific enquiry has already been mentioned. But it can also serve other purposes. Thus, it can provide an excellent opportunity for consolidation and revision. It can also enable students to work out hypotheses for themselves through a mutual exchange of ideas under the guidance of the teacher. Perhaps the most essential prelude to a successful discussion is adequate preparation of the material to be discussed, both by the teacher and the pupils (perhaps in preparation). The teacher will need to have a broad idea of the path the discussion is likely to follow and to have formulated in advance the sort of opening questions that are likely to set it off on the right track. Teachers should take care, however, lest too much advance preparation should cause a potentially fruitful discussion to develop into a 'set-piece' affair of limited educational value.

Class practical work and demonstration

In this course a distinction has been drawn between demonstration by the teacher and the practical work carried out by the pupils. In class practical work the accent is either on *using* information, techniques, and concepts or *working them out*. It is essentially an investigatory or problem-solving activity.

A demonstration can also serve this purpose to some extent, particularly if the level of sophistication of the subject matter is relatively high. For instance, a demonstration by the teacher, aided by pupils' comments, may well give a better understanding of the controls in a particular experiment than if the pupils were left to their own devices.

In the main however, demonstration is a method of imparting a piece of information, describing a concept or technique, or introducing a point for discussion, without the trial and error confusion that invariably plays a part in class work. A topic demonstrated may, or may not, be something the pupils could have investigated themselves.

The evidence available suggests that for conveying information and describing concepts and techniques, a demonstration is as efficient as, if not better than, class work. Furthermore it is much quicker and, if used judiciously, can gain time in the course for more class practical work.

There is a place in biology teaching for both demonstration and class work. They are complementary to each other.

Group practical work

For the majority of their class work pupils will be working in small groups all performing the same investigation. But on occasions the work can be so arranged that each group is concerned with a different task; for instance when we wish to show that a particular concept applies to a whole range of organisms or when pupils are involved in a variety of different problems or investigations. The organization of such practical work differs little from normal routine, except that when the work is completed, it is important that the class as a whole should be made aware of the findings of the different groups. In addition, it is a good idea to choose a member of each group to give an account of their work. The results of each group investigation should be summarized on the board so that the whole class can make deductions from them.

Even when groups of pupils are all undertaking the same investigation it is wise to put a summary of the results on the board and to hold a discussion. In this way many important points, such as the purpose of controls, experimental error, and the making of hypotheses can be introduced. Class average results are far more significant than those obtained by single groups.

Long-term investigations

Some of the investigations undertaken during this course last two or three weeks or even longer. They need not occupy the whole time, however, and the art of using them for teaching is to integrate them with other work. To obtain the best value from such exercises, students should feel that they are individually responsible for their investigations throughout the whole period of their existence. This necessitates an allowance of time during class periods to make records and carry out any procedures needed to keep the experiments going. The natural tendency is to forget them once they have been set up, or to regard them as something of secondary importance, peripheral to the usual short-term class work. Here again, discussion will play an important part at the conclusion of the work so as to ensure that the main principles and implications of the investigation are not obscured by minor problems in experimental technique or other irrelevant difficulties.

Writing-up practical work

There are many different ways of writing-up practical work and all of them have their merits. It would be wrong to assert dogmatically that any one is the best. The following suggestions are intended to provide a rough guide which can be adapted to suit individual preferences.

a. When writing up an investigation a pupil should aim to give in his own words a concise account of everything he did and why he did it. The use of distinct headings has much to commend it, particularly for juniors. These could be – problem, hypothesis, experimental method, results, deductions, suggestions for further investigations.

b. Some experiments involve relatively complicated procedures. We find that pupils can benefit in their understanding if they are required to summarize the method in a brief statement or 'outline' – which may be only a few sentences long – before becoming involved in technical details which can obscure the overall pattern of procedure.

c. It is not always necessary to write up each piece of work separately and completely. Where appropriate, summaries of a group of related experiments can be written up together.

d. With reasonable guidance, pupils should become capable of writing up their practical work unaided. Dictated notes will seldom be needed except possibly to supplement the instructions for experimental procedure included in the text.

e. Pupils will find it helpful to cross-reference their own note-books to the appropriate portions of the text, particularly where practical work is involved. Such a practice greatly simplifies the problems of revision.

f. The role of drawings and diagrams in biology can be greatly over-emphasized. We suggest that a drawing is only needed (*1*) to explain a point which cannot be adequately and concisely described in words, and (*2*) when a matter of structural detail either in an organism or a piece of apparatus needs careful exposition. In such instances a large, clear drawing can be a valuable aid to understanding. Small sketches and simple schematic diagrams should also be encouraged but only where they clarify a situation which is inadequately covered by a verbal description.

Visual aids

A number of 8mm Film Loops have been prepared in connection with the course. They are closely integrated with particular sections of the text. Each loop is contained in a plastic cassette and is intended for use with projectors such as the Technicolor 800E. The Film Loops are all silent but detailed teaching notes are provided with them, explaining their relevance to the course and giving an outline of the sequence of shots. Suggestions are also made regarding topics for class discussion which might be pursued after the showing of the films. The Film Loops can be classified roughly into four kinds:

a. Those dealing with particular topics where a dynamic process is involved which cannot be readily observed by a class in the laboratory, for example, the feeding mechanism of a housefly (Year III) and the fertilization of an egg by a sperm (Year I).

b. The explanation of techniques such as the use of the microscope (Year I) or the squash method of making a chromosome preparation (Year V).

c. Those showing the sequence of an experiment which could not be carried out in a school for example the uptake of ^{14}C by *Chlorella* (Year III).

d. Those showing a chain of living events such as a food chain (Year II).

The 800E projector operates by back projection, requires no black-out, and automatically repeats the showing of a film. Loading simply consists in placing the film cassette in a slot. In such circumstances, film showing can become a much less formalized affair than in the past, and if the projector is placed on a side bench small groups of pupils can view a film while the rest of the class is engaged in some other activity. This is particularly useful where practical techniques are involved; students can quickly consult the film when they are in difficulty.

16mm sound films are also recommended in the guide for some purposes but none have been produced specifically for the Nuffield Course. The 8mm Film Loops are intended to be an integral part of the teaching and to pose problems which can stimulate discussion between teacher and class. If 16mm sound films are used with this course it is essential that they should also meet the same requirements.

A small number of 2 × 2 inch transparencies, some separate pictures, and a sound recording have also been produced in connection with the course. These are referred to at appropriate points in the guide. All these aid materials can be obtained through the same sources as the texts and guides.

Teaching elementary fieldwork (Years III and IV)

a. Is fieldwork really necessary?

We hear many statements these days attempting to justify the inclusion of fieldwork in an elementary course: that it promotes a love of nature; that it enables children to see and handle living things; that it develops powers of observation; that it satisfies an acquisitive instinct; and so forth. Such arguments surely overlook the main issue. Among the important general principles in biology are those concerning the behaviour of *populations* and the relationships of the plants and animals which compose them. The principles of spatial distribution, succession, competition, and food-chains, to mention just a few, are fundamental to our understanding of the subject at any level. Moreover, they are aspects which, in the last resort, can only be studied satisfactorily in the field. Either we must include them, and in so doing concede the importance of fieldwork; or we must disregard them altogether. There seem to us to be no half measures; and for this reason, we have adopted the first policy unreservedly.

b. The selection of organisms and sites for study

One of the superstitions that has adversely affected the teaching of fieldwork in schools is the belief that it is necessary to study a 'well-defined habitat' – whatever this may mean. Unfortunately, such an idea has been perpetuated in some examinations. All too frequently, the result is a decorative map and a long list of names, neither of which is likely to contribute much of value to biological understanding.

The present course in ecology attempts to break new ground in two respects:

1. It is so closely integrated with the relevant work in physiology and behaviour that it is sometimes difficult to discern where one ends and the other begins.

2. It is concerned with illustrating certain clear-cut ecological principles.

In designing our plan to work, we have been acutely aware of the wide variety of circumstances for which we must provide ranging from luxuriant conditions of the South to extreme industrialization in the Midlands and North. Our plan has therefore been to state clearly the principles we wish to illustrate, then to devise simple experiments which, we hope, the great majority of schools will be able to carry out, even under the most inimical conditions.

The guide contains a number of suggested alternatives, both in the selection of organisms and methods of working, which might be used, should the ones given in the text prove inappropriate. A resourceful teacher will devise alternatives of his own. For schools in country districts, there will clearly be a wide choice of materials, any of which might be considered. At the opposite end of the scale, it may well be that there are schools where the local conditions are such as to defeat the ingenuity of even the most adaptable teacher. The solution then would seem to be periodic planned excursions.

The flexibility of material, and the variability of local conditions, preclude the anticipation of specific, hard-and-fast results. We have a situation which is essentially one of research, the students and teacher working together as a team, pursuing a line of enquiry and maintaining an openminded, critical approach. The fundamental point to bear in mind is that we are concerned, neither with specific organisms nor with rigidly prescribed experiments, but with *principles*. How these principles are set forth to students must be left to the discretion of the teacher. From time to time, seasonal considerations necessitate breaks in the smooth continuity of the course. The very nature of fieldwork makes this unavoidable. The fact that such breaks occur has its value in training students to be aware of the part played by the seasonal rhythm in the lives of animals and plants.

We hope that a clear statement of aims, and suggestions for practical work, both in the guide and the text, may assist teachers to exploit their opportunities and to carry out careful planning in advance.

References and indexes

For ease of reference the guide chapters bear the same main headings and numbers as those in the texts. Separate indexes will be found at the back of every guide, except Teachers' Guide Year V which contains a comprehensive index covering the five years of the course.

Chapter 1

Man and microbes: the discovery of small organisms

Objectives

The theme for this term's work is 'the world of small organisms'. Although we shall be concerned chiefly with bacteria, mention will be made of other micro-organisms as well. This is a subject which lends itself rather well to a semi-historical treatment. Starting with the discovery of microbes by Leeuwenhoek and his construction of the microscope, we follow this with the work of Spallanzani and early experiments on bacteria, some of which can be repeated in the laboratory. The work of Pasteur provides a splendid example of experimental design and links with modern applications of his findings. The work of such men as Koch, Lister, and Jenner introduces the modern side of bacteriology, particularly as applied to man and his everyday needs.

The discovery of microbes

The early work on micro-organisms dating from the construction of his own microscopes by Leeuwenhoek provides a good illustration of the way in which one scientific discovery can lead to the next. The purpose of this chapter is to show the sort of problems the experimenters in the seventeenth and eighteenth centuries had to overcome – not least the existence of a theory of spontaneous generation which was contrary to their findings in the laboratory. Later in the chapter students are introduced to micro-organisms in the air and carry out investigations on them.

The order of development of the chapter is as follows:

1. Investigating small organisms involves the use of a lens.
2. Simple lenses were made by Anton van Leeuwenhoek in the seventeenth century by means of which he was able to see small organisms.
3. The theory of spontaneous generation – that living things arose from non-living material – was a stumbling block in the way of early scientists.
4. Lazaro Spallanzani failed to perform a convincing experiment to disprove this theory.
5. Some micro-organisms are harmful while others, such as yeast, are useful to man.
6. Micro-organisms are of different shapes and sizes.
7. Micro-organisms are everywhere in the air.
8. Pasteur disproved the theory of spontaneous generation by

showing that if micro-organisms in the air are excluded from a sterile broth, the broth will not go sour.

Suggestions for practical work

	Section		Page Text	Page Guide
Class practical work	1.1	The use of a hand lens	1	3
	1.15	What happens when yeast and sugar are mixed together and warmed?	6	7
	1.3	Micro-organisms in the air	13	11
Demonstrations	1.14	Looking at yeast under the microscope	5	7
	1.15, 1.16, and 1.3 could all be used as demonstrations if necessary, but it is to be hoped that at least some (and preferably all) experiments would be carried out by the class			
Optional practical work	1.16	Making bread	8	8
	1.17	Making your own wine	9	9
	1.22	Micro-organisms in the soil	11	9

Before attempting the practical work of this chapter, it might be as well to refer to section 2.11 which deals with microbiological techniques in some detail. A list is given below of the basic apparatus and materials required by a class of thirty pupils for the term's work:

Apparatus

1 × 6 inch boiling tubes	1 gross
$\frac{3}{4}$ × 5 inch test-tubes	1 gross
Penfiller type pipettes, 2 cm^3 and 10 cm^3 plastic disposable syringes	5 dozen
3 mm diameter glass tubing	
Nichrome inoculating loops	2 dozen
McCartney bottles	2 dozen
Chinagraph pencils	
Disposable Petri dishes	Box of 200

Media etc.

Nutrient Agar	3 bottles of tablets
Sabouraud Maltose Agar	1 bottle of tablets
Nutrient Broth No. 2	3 bottles of tablets
Blood Agar Base	3 bottles of tablets
MacConkey Agar	3 bottles of tablets
Penicillin discs (1·5 units)	4 bottles of tablets
Penicillin discs (5 units)	5 bottles of tablets
Resazurin	10 tablets
Streptomycin (25 μg discs)	1 bottle
Bacillus subtilis (impregnated discs)	1 bottle
Escherichia coli (impregnated discs)	1 bottle
Staphylococcus albus (imgregnated discs)	1 bottle

Cultures
Penicillium sp.

Prepared microscope slides
Yeast cells
Penicillium sp.
Bacillus megaterium

Reagents and chemicals
Phenol
Ammonium Sulphate
Potassium dihydrogen phosphate
Dipotassium hydrogen phosphate
Dextrose
B.D.H. Universal Indicator
Brom-cresol-purple
Lysol

Other materials

Dried yeast	1 tin (not a packet)

Introduction

In the past, the study of microbiology has often been carried out only as a sixth form project. It is, therefore, a rather revolutionary step to introduce it so early. There are several reasons for doing so. Much of this introductory course is concerned with man and his importance as part of the living world. The picture would obviously be incomplete without some reference to his health which, in turn, brings in reasons for hygiene and cleanliness.

The experimental work of the course centres largely upon showing the widespread occurrence of micro-organisms in the laboratory and the immediate field of the pupil's experience, such as the preparation and storage of food in the home, personal cleanliness, and so forth.

Visits to milk and cheese factories, breweries and food-canning firms are important in linking in the pupil's mind studies made in school with the world around, and serve to emphasize the importance of the practical work undertaken in class.

It is not beyond the skill of second year pupils to master simple bacteriological techniques and to appreciate the reasons underlying them. In attempting such a scheme, however, it is essential that the concepts should be chosen carefully and that they should be interpreted practically, in ways which are not above the level of sophistication of twelve year olds.

1.1
The use of a hand lens
Notes on teaching

There is a large jump from the kinds of living things considered experimentally in the previous year – earthworms for example – to the world of micro-organisms, and students will need plenty of time and opportunity for discussion to adjust their outlook to a microscopic world. Success in this early section of the course will depend upon an appreciation of relative sizes and, for example, an idea of just how small a microbe really is. Coupled with this is the idea of the need for magnification. Here is a good opportunity for revising the use of the hand lens and for emphasizing once again that there is a right and a wrong way of using it.

A hair is more easily held in the hand for focusing if placed across a small square of Sellotape.

When using hand lenses, students should be encouraged to say what difficulties they encounter, such as keeping the lens still. If possible they should have opportunities of using both × 5 and × 8 lenses, so that they can appreciate the significance of a reduction in the working distance as the magnification increases. An understanding of the use and limitation of a hand lens leads us logically to a discussion of Leeuwenhoek's work. After all, his microscope was nothing more than a single mounted lens of short focal length.

1.11
The first microscope
Historical notes on Anton van Leeuwenhoek (1632–1723)

The son of a basket maker, Leeuwenhoek spent most of his life in the Dutch town of Delft. He was a draper by trade but reserved his energies for his hobby of grinding lenses and making microscopes. During his lifetime he made about 247 instruments. It was through the construction of microscopes that Leeuwenhoek was led to make investigations of his own on micro-organisms. His microscopes were really no more than simple magnifying glasses consisting of a brass plate with a small lens let in at one side (see text figure 2). The object to be examined was impaled on a sharp point which, by means of a delicate ratchet screw, could be raised and lowered and moved nearer or farther away. By holding the brass frame up to the light, his lenses gave a magnification of up to 240 diameters.

By means of these simple microscopes, Leeuwenhoek examined a number of objects from rain drops to particles of sand, pond water, and even pepper grains. For some of his work he must have used a form of reflected light and dark ground illumination for he was the first person to see and draw bacteria. He called them his 'little animals' but never connected them with disease. He also made the first scientific attempt at estimating the micro-population of a drop of water and the first measurement of blood corpuscles. On his death most of his microscopes were sold; at his request twenty-six were sent to the Royal Society in London.

1.12
Where do microbes come from?
Notes on teaching

The story of the theory of spontaneous generation and its eventual disproof is fascinating, but we must be careful not to be carried away into a historical ramble which is largely irrelevant to the purpose of this section. The point to be stressed here is the imperfection of these early experiments. Spallanzani had the right idea and took a big step towards showing that living things cannot arise from the dead. But it is significant that few people in his time were convinced by his experiments and arguments. No doubt there were many reasons for this. One certainly was that Spallanzani was thinking well beyond the climate of opinion of his age. People were not yet in the right frame of mind to receive and ponder on this new outlook. Another reason for Spallanzani's lack of impact on the public mind no doubt lay in the nature of the experiments themselves. Conditions inside a sealed flask are very different from those in the air outside. Could it not have been that microbes failed to grow because of the changed conditions resulting from boiling the broth?

We do not know whether Spallanzani used controls; perhaps he did. The obvious control for his nineteen boiled and sealed flasks would be an equal number of unboiled ones. What would have happened in them? Another possibility would be to have a number of boiled flasks open to the air. These would, of course, have become colonized by micro-organisms directly they cooled down. At this point we can ask the question: Could we not design an experiment to let air into a sterilized flask but to exclude the micro-organisms? Could we use a filter? This brings us to the standard practice in laboratories today of putting a cottonwool bung in sterilized tubes and flasks, and also in those containing pure cultures of bacteria.

After performing the experiment using yeast in section 1.15, the class is asked to refer again to this section (1.12) and to offer reasons for Spallanzani's failure to convince the public of his time.

Spontaneous generation

The idea that living things can be formed directly from non-living materials dates back to the time of Aristotle (384–322 B.C.). He believed that many small organisms were formed in this way; for instance, that aphids (greenfly) arose from the dew which forms on the leaves of plants, and that fleas developed from putrid matter such as dung. This conception, supported by respect for Aristotle's authority, played an important part in medieval scientific thinking. It was challenged, however, in 1650, by the Italian Redi, who suggested that 'worms' found in the dead bodies of eels were really a stage in the life history of flies which had laid their eggs on the bodies of the fish. He was perfectly right. In an endeavour to use this idea for the disproof of the former theory, he set up four flasks containing rotten meat which he sealed, and four more which he left open. He then observed the results. What would they have been? This is a good question for discussion in class.

Here is an early attempt at the design of a controlled experiment. The experimenter was attempting to study the effect of one variable – air. But by sealing half the flasks, he not only kept out the flies which might have laid eggs but he also excluded the air, and in this respect his experiment was open to the same objections as that of Spallanzani (see section 1.12).

Spallanzani (1729–99) knew that, in order to destroy any micro-organisms present it was necessary to boil an infusion for forty-five minutes. He had no knowledge of bacteria as they are thought of now, and his 'animalculae', covered a wide range of organisms including fungi and small insects, and mites. But in spite of the crudeness of his methods, the conclusions reached by Spallanzani were almost identical with those of Pasteur nearly a century later. The difference between them lay in the refinements of Pasteur's experimental techniques, and the way in which he made use of controls.

The story of the disproof of spontaneous generation runs through the scientific work of the seventeenth and eighteenth centuries. Men like van Helmont, Redi, Spallanzani, and others all made their several contributions which depended largely on gradual improvements in apparatus and in the technique of designing scientific experiments.

1.13

What are microbes?

Notes on teaching

In the eighteenth century the word 'germ' was used to describe a whole assemblage of living organisms which were invisible to the naked eye. In addition to those forms which we now class as micro-organisms, men like Spallanzani would almost certainly have seen many small insects in their immature stages. This would explain why he talked about 'little animals', suggesting that he was particularly struck by their ability to move about.

Today we regard micro-organisms as being either bacteria, viruses, fungi (e.g. unicellular forms such as yeast), protozoans or algae. In class the question will almost certainly arise as to whether such things are plants or animals. We need not pursue the question very far now and we should avoid expounding the differences dogmatically. The children should be encouraged to form their own judgment at this stage and here is a good topic for discussion in class.

Use of microscopes

It is not envisaged that the class will ever need to use a microscope on their own, but the more a microscope can be used for demonstration purposes, the better. On the whole, microprojectors are not recommended because of the relatively low resolving power of most instruments. An ordinary school microscope with × 10 eye-piece and $\frac{2}{3}$ inch (× 10) and $\frac{1}{6}$ inch (× 40) objectives with an Abbe condenser is preferable.

Micro-organisms are very easy to mount for examination under low or high power. Usually a satisfactory method is simply to transfer a small amount of culture with a mounted needle to a drop of water on a microscope slide. Stir well to mix thoroughly and then apply a cover-slip. Dry off any excess water round the edge of the cover-slip with a piece of blotting paper. Under high power (about × 400) it should be possible to get quite a good idea of the shapes and sizes of most bacteria.

Permanent preparations of bacteria can be bought from dealers and it is certainly worth while to have a few examples of forms which cause disease in man. An alternative source of supply is the pathological laboratory of any large hospital, where great numbers of these slides are made as a routine. Frequently, the chief technician will be only too glad to get rid of them once they have served their purpose. They are quite safe to handle.

Microbes 'good' and 'bad'

When teaching this and subsequent sections of the term's work, it is important to bear in mind the popularly held misconception that all microbes are 'bad'; that is to say, they either cause disease or inflict some other kind of harm. This is an attitude which is perfectly understandable for the most sensational applications of microbiology are devoted almost exclusively to the conquest and prevention of disease in some form or other. This is a notion spread by the newspapers and television.

We hope that by introducing bread making at this stage, and also by the production of alcohol by yeast, we may be able to some extent to correct this distorted viewpoint (see section 1.16).

It is too early yet to consider more sophisticated topics such as the nitrogen cycle. But we have already been involved with decay and the fact that dead plants and animals rot in the soil, forming humus. We may well pose the question in class – how do you think this rotting process is carried out? Suppose we can put forward some hypothesis to explain it, how could we set about testing it? Other examples of 'good' microbes (*Penicillium*, for example) occur in later chapters.

Before beginning experiments using yeast, it may be as well to have a look at actual yeast cells under the microscope. This will probably best be done as a demonstration, using a stained yeast culture.

1.14
Microbes useful to man – yeast
Notes on teaching

Making a yeast culture

Yeast can usually be obtained in the live state either from bakers or from a brewery. Alternatively it can be purchased at grocers in dried form. A good culture medium for yeast can be made by dissolving 2 g malt extract in a small beaker of warm distilled water and making this up to 100 cm^3 (i.e. a 2 per cent solution). Place in a 250 cm^3 flask and add about 2 g powered agar. Sterilize in an autoclave and allow to cool, then inoculate with yeast culture. To one drop of yeast culture add one drop of Cotton Blue Lactophenol. The yeast cells can then be seen clearly under high power. Alternatively they can be examined under a × 100 lens using dark ground illumination.

Methods of sterilizing apparatus etc. are described in guide section 2.11.

1.15
What happens when yeast and sugar are mixed together and warmed?

Apparatus required per class

1. 8 g fresh bakers' yeast or 4 g Allinson's dried yeast (from a tin) dissolved in 100 cm^3 10 per cent glucose solution

2. 8 g fresh bakers' yeast or 4 g Allinson's dried yeast dissolved in 100 cm^3 distilled water

3. 100 cm^3 10 per cent glucose solution.

Water bath set at 37°C

Per group or per working pair

2×6×1 inch test-tubes filled two-thirds with solution (*1*) above (A) and (B)

1 × 6 × 1 inch test-tube filled two-thirds with solution (*2*) above (C)
1 × 6 × 1 inch test-tube filled two-thirds with solution (*3*) above (D)
°C Thermometer
4 rubber balloons

Notes on teaching

The object of this experiment is to establish that when yeast and sugar are mixed together, a gas is given off. Moreover, the rate at which it is evolved is governed by the temperature. This sets the stage for the next experiment on bread making, where the findings are put to practical use.

Incidentally, this experiment gives a good opportunity for emphasizing the importance of controls and the importance of studying only one variable at a time. Discussion of the influence of temperature should be encouraged before stage (*5*) of the practical is begun.

An important question of principle arises here – should we say what the gas is? If we introduce the limewater test and establish that we have got CO_2 what is the justification for doing so? It is a little like producing a rabbit out of a hat. Moreover, a number of very awkward questions follow, for instance – where does the carbon come from? This lands us in all sorts of difficulties connected with the chemical composition of food which should be avoided at this stage. For the present purpose and for the success of the next experiment, it does not matter in the least what the gas is. If children ask, we can certainly say it is CO_2 and leave it at that. It may well be that they will have some idea already from their chemistry, in which case, so much the better.

Notes on the conduct of the practical work

1. Fresh yeast gives more reliable results in more limited time. But if dried yeast is used, that made by Allinson Ltd in a tin (not a packet) is best. Note that only half as much dried as fresh yeast should be used.
2. For tubes A and B mix sufficient of the yeast and glucose solution to supply the whole class in a flask half an hour beforehand. Shake at intervals to ensure that the yeast cells have become fully hydrated. If this procedure is adopted, the balloon on tube A should inflate within fifteen minutes at 37°C.
3. Blowing up and collapsing the balloons beforehand allows them to inflate more readily during the experiment.
4. By using test-tubes instead of round flasks, all those which are to be heated can be placed together in a rack using only one water bath for the whole class.

1.16
Making bread

Apparatus required per group of four or six
150 g plain flour
2 g dried yeast (Allinson's)
4 g sugar
100 cm^3 water warmed to about 32°C
Mixing bowl
3 × 1 lb jam jars or 3 large evaporating basins
Chinagraph pencil

Notes on teaching

The experiment on baking follows logically from the findings in section 1.14 – that a gas is given off when yeast and sugar solution are mixed and that the rate at which it is produced depends upon the temperature. It might be useful here to discuss just how the gas evolved causes the dough to rise. Why does dough 'prove' more quickly when it is warm? There is no reason why, if an oven is available, the dough should not be baked. If this is done, it would be best to place the kneaded dough in containers such as cocoa tins rather than in jam jars. A comparison of the resulting 'bread' can be made by feeling the textures, cutting up to show internal structure and, most important of all, by eating.

A point well worth discussion is why the dough does not continue to rise after being placed in the warm. This could lead to further considerations of what yeast really is – a living organism which is presumably killed at high temperatures. This would be a useful prelude to experiments in section 1.3 which involve sterilization of a culture medium by heating in a pressure cooker or autoclave.

1.17
Making your own wine

Apparatus required per pair

Apple juice
Dried yeast (Allinson's)
2 test-tubes and cottonwool plugs

Notes on teaching

We have included wine making as an optional practical which could be done if time permitted. Incidentally, this is something which could quite easily be attempted out of school or at home as a 'project'.

1.2
Bacteria

Notes on teaching

This section deals with bacteria in a purely factual way. The intention is to provide sufficient background information for an understanding of the rest of the chapter. No doubt teachers may wish to extend their knowledge of bacteria and a number of references to works of a general and specialist character are included in the bibliography at the end of this chapter.

1.22
Micro-organisms in the soil

Apparatus required

per class	*per working pair*
3 or 4 microscopes	1 flask containing soil solution
Incubator at 37°C	1 tube containing 5 cm^3 Blood Agar Base
	1 Petri dish
	1×2 cm^3 pipette or 2 cm^3 disposable syringe
	1 mounted needle, microscope slide and cover-slip

Notes on teaching

The practical work of the next section (1.3) shows that micro-organisms exist in the air, but since one of the objects of this chapter is to stress their ubiquity, this optional experiment is included to show that micro-organisms also exist in large numbers in any sample of soil. Colonies of bacteria should develop on the agar within twenty-four to forty-eight hours at 37°C which is usually too high a temperature for the quick development of moulds. The Petri dishes can be removed from the incubator when the colonies have developed and if immediate examination is not possible the development of moulds can be inhibited by placing a circle of filter paper, onto which a few drops of 40 per cent formalin have been pipetted, in the lid of the Petri dish.

The procedure for making the soil solution is to shake up 10 g of garden soil in 90 cm^3 distilled water and to transfer, by means of a pipette, 1 cm^3 of the suspension to 99 cm^3 distilled water to give a dilution of 1/1000.

Blood Agar Base is obtainable from Messrs Oxoid Ltd, Southwark Bridge Road, London, S.E.1, in tablet form. Since 1 cm^3 soil inoculum is to be added to the agar, this volume must be subtracted from the volume of distilled water required for the reconstitution of the medium, otherwise the gel strength of the agar will be weakened. Hence to two tablets add 9 cm^3 of distilled water and soak for fifteen minutes. This is then autoclaved for fifteen minutes at 15 lb/in^2. Each Petri dish needs 9 cm^3. It will be easier to autoclave the 9 cm^3 amounts in separate test-tubes as one batch. Then cool to 45°C. They will then be at the right temperature for plating out by the class. The members of the class will probably find it easier to use a 2 cm^3 disposable plastic syringe for the addition of the soil inoculum, than an ordinary 1 cm^3 pipette.

Alternative experiments

a. Culturing micro-fungi in the soil

Micro-fungi are present in any sample of soil and can easily be cultured in the same way as soil bacteria from a solution of soil. Malt Agar, also obtainable from Oxoid Ltd, is used as the culture medium instead of Blood Agar Base. After plating out, the Petri dishes should be left at room temperature (25°C) for four or five days. Fungi form a mycelium which under the microscope can be seen to be composed of a mass of hyphal threads.

b. Culturing Pythium *on cress seedlings*

The damping-off fungus, *Pythium*, usually appears among cress seedlings grown in very damp, unsterilized soil. It attacks the hypocotyl region of the seedlings, causing them to bend over and finally to wither.

The class could grow cress seedlings in very damp soil in plant pots enclosed in Polythene bags, and placed near a source of heat such as warm pipes. *Pythium* is almost sure to occur in several of the cress cultures and its effects on the seedlings can be noted. The class can then be asked to suggest where *Pythium* comes from – air, water, soil, or the cress seedlings themselves? Experiments can then be devised to test these theories. Working in groups, each group is provided with sterilized soil, water, cress seeds and jars or plant pots. Each group sets up one culture using the sterilized soil as a control and this is compared with cultures in which there is one non-sterile factor. In each case the number of seedlings which are infected with *Pythium* in a given culture is determined. A squash preparation of *Pythium* could be made by mounting a portion of the hypocotyl region of an affected seedling in water and applying gentle pressure to the cover-slip. Examination should show the hyphal threads of the fungus penetrating the plant tissues.

Numbers of bacteria in the soil

Calculations have shown that the top six inches of soil contain about 1,500 lb of bacteria per acre, equivalent to 2,500 million bacteria per g of soil. A saltspoonful of dry garden soil weighs about 1½ g, yet this quantity contains roughly as many bacteria as there are human beings on the earth.

1.3
Micro-organisms in the air

Apparatus required per set
Autoclave or pressure cooker

Per pair or group of four
5×¾ inch test-tubes
Cottonwool and tinfoil
1×3 inch length of fine glass tubing
1 S-shaped length of fine glass tubing
50 cm³ Nutrient Broth No. 2

Notes on teaching

The story of Pasteur's disproof of the theory of spontaneous generation is treated fairly fully in the text and needs no elaboration here. For further reading, see the bibliography. This section provides ample opportunities for class discussion centred round the design of experiments, the use of proper controls and so forth. Why did Pasteur succeed so brilliantly in his attack on the idea of spontaneous generation, when Spallanzani had been less successful?

The final experiment is intended to give some idea of the sort of techniques and procedures which Pasteur employed. Notice that we use broth (as Pasteur did), not agar plates. The agar method of culture was not developed in Pasteur's day.

This experiment (section 1.3) should be conducted as a class practical, the intention being to give pupils an opportunity of experiencing the problems facing the scientist who studies bacteria. For instance, they should all have an opportunity of using a pressure cooker or autoclave for sterilization. If possible, the class should work in pairs but if apparatus does not allow for this, groups should not be larger than four.

Blood Agar Base can be obtained from Oxoid Ltd (see above), in tablet form. One tablet is added to 10 cm³ distilled water. Using ¾ inch test-tubes, 10 cm³ nutrient broth per tube should be sufficient. Approximately twenty-five test-tubes of this size will fit into a high dome pressure cooker. The standard size of bacteriological test-tubes is 6×1 inch with a capacity of 60 cm³. If these are used, more nutrient broth per tube will be required and only nineteen tubes will fit into the ordinary size of pressure cooker.

The glass tubing to be used should have an internal diameter of not more than about 3 mm.

It may be best to summarize class results on the board, so that they can be compared and discussed.

Film strip

a. There is quite a useful film strip entitled 'Pasteur and Microbes' produced by Common Ground, 44 Fulham Road, S.W.3.
b. An 8 mm film loop in colour, entitled 'An experiment of Louis Pasteur', No. BB/278, with a running time of 2 minutes 30 seconds, is obtainable from Gateway Film Productions Ltd, 470 Green Lanes, Palmers Green, London N.13. The film shows an experiment, using swan-necked flasks. By tilting one flask, the broth it contains is brought into contact with bacteria trapped in the bend in the neck and turns turbid. The broth in the other flask, which is not tilted, remains clear.

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Further reading

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Chapter 2

Growing bacteria

Objectives

The subject of bacteria culture follows on logically from the discoveries of Pasteur considered at the end of the previous chapter. The purpose of this chapter is first to show how the principles developed by Pasteur were applied by Koch and others, and resulted in the methods of growing pure strains that are used today. The second aim is to give students an opportunity of trying out these methods for themselves under laboratory conditions and to show how they are applied to the solution of some common problems in bacteriology.

The order of development of chapter is as follows:

1. The researches of Robert Koch resulted in the perfection of methods for obtaining pure cultures of bacteria.
2. Large numbers of micro-organisms exist in the soil and air and can be cultured on nutrient media.
3. Milk contains bacteria.
4. Through his researches in connection with the wine industry in France, Pasteur evolved a method of destroying bacteria by heating wine to 60°C. This was later called 'Pasteurization'.
5. Pasteurization of milk results in better keeping qualities.
6. The degree of freshness of milk can be tested by means of the dye resazurin.

Suggestions for practical work

	Section		*Page Text*	*Guide*
Class practical work	2.12	Growing bacteria from the air	20	19
	2.13	Bacteria on ourselves	21	20
	2.2	Are bacteria present in milk?	22	20
	2.22	Can we improve the keeping qualities of milk by pasteurization?	24	22
Demonstration	2.23	Testing milk for freshness (the resazurin test)	25	23
Optional practical work	2.12	Growing bacteria at different temperatures	20	19
	2.13	Bacteria on ourselves (finger prints after drying hands on a dirty towel, a paper towel, and in hot air).	21	20

2.1 Early discoveries about growing bacteria

Notes on teaching

The work of Robert Koch was, essentially, a logical extension of the work of Pasteur, Spallanzani, and others before him. The earlier workers, Pasteur among them, were experimenters in the true sense of the word. As knowledge and experience increased, so their technique became better, reaching its climax in the beautifully designed experiments of Pasteur in connection with the theory of spontaneous generation.

By contrast, Koch was much more of a technologist – and a very brilliant one at that. While he made extensive use of earlier discoveries, much of his early work was conducted out of contact with the great scientists of his day. It is this, perhaps, which accounts for the originality of many of his discoveries. As far as this course is concerned, there are two particular points that are worth stressing in relation to Koch's discoveries. The first is the method of growing bacteria on agar plates – used for the investigations in the later part of this chapter and throughout the laboratories of the world today. Secondly, as we saw in the last chapter, earlier workers with the partial exception of Pasteur, paid little attention to the kinds of bacteria found in their cultures, which therefore tended to contain an assortment of different forms. Koch realized that the time had come to put bacteriology on a more systematic footing and it is to him that we owe the technique of growing pure strains. Again, Koch's techniques are basically those used today.

Notes on Robert Koch (1843–1910)

After a medical training at Göttingen, he eventually became Professor of Medicine in Berlin (1885), and later was appointed Director of the Institute for Infectious Diseases (1891). Among his many discoveries, he was responsible for isolating and culturing the anthrax bacillus (discovered in the first place by Pasteur), and he devised a method of inoculating cattle against the disease. He also discovered, isolated, and cultured the bacillus responsible for tuberculosis – a disease which was common in his day. He was awarded the Nobel Prize for Medicine in 1905. Much of his success was due to improvements in microscopy which he carried out, both in the instruments themselves and their use, and also in the reagents used for treating and mounting micro-organisms to make them more visible. For instance, it is to him that we owe the introduction of aniline dyes in staining (e.g. aniline blue).

2.11 Microbiological techniques

Before beginning a practical study of micro-organisms, it is important that students should understand that certain elementary precautions must be observed. There is no need to be alarmist here, but since the cultures they will obtain are of virtually unknown composition, it is by no means out of the question that certain pathological forms may be included among them. This possibility is unavoidable, but the chance of infection is negligible, provided a few commonsense steps are taken to avoid it.

The following suggestions may be a help in providing general guidance to microbiological techniques.

The methods are given in some detail, not because they are in any way complicated, but in order that difficulties may be avoided which, from experience, are known to be possible.

Success depends upon:

a. Using the correct culture medium
b. Proper culture conditions
c. Prevention of entry of contaminants
d. Proper sterilization

1. Sterile conditions in the laboratory

Since one of the aims of this course is to show the connection between cleanliness and the spread of bacteria, sterile laboratory conditions should be encouraged, so far as it is humanly possible. Swabbing down benches with 10 per cent Lysol or another similar disinfectant before the class, insistence on hand-washing, not breathing over cultures, and the proper disposal of cultures at the end of the class, will cut down any small risk there might be in culturing organisms amongst which there might be pathogens. It should be emphasized, however, that there is probably no more risk of infection from these sources, than from the ordinary day-to-day hazards of the biology laboratory.

2. Sterilizing equipment

A proper autoclave is, of course, the best means of sterilizing, but an ordinary domestic pressure cooker, of the Prestige type, can equally well be used. Preferably it should be at least 8 inches in diameter with a high dome lid.

All glassware must be sterilized. This is usually by dry heat in an oven at 160°C. The temperature must not be allowed to rise too rapidly, otherwise the glassware may crack. Keep the oven door closed while sterilizing and until the glassware is cold. Small, relatively cheap ovens are available which are quite suitable for this purpose.

Glassware can also be sterilized in a pressure cooker (at 15 $\mathrm{lb/in^2}$ for twenty minutes). To avoid getting dishes wet inside, they can be wrapped in aluminium foil, or even in several layers of newspapers.

Packs of sterile transparent plastic Petri dishes are now available in lots of ten, sealed in a Polythene bag. Although they are cheaper than glass Petri dishes, in the long run they prove more expensive, since, once used, they cannot be re-sterilized.

3. Culture media

Choice of medium depends upon the organisms to be cultured. Generally speaking, fungi prefer more acid and bacteria more alkaline conditions for growth. By selecting a medium whose pH is outside the range of most fungi, the bacteria will grow while the growth of fungi will be suppressed. This is a distinct advantage when dealing with bacteria, since their colonies can be greatly obscured by the growth of moulds.

Culture media can be liquid or solid and can be purchased ready made up and sterile, in culture bottles or in Petri dishes. They can also be purchased in powder or tablet form. For the purpose of this course, most of the recommended media can be purchased as tablets, and it would probably save time to do so. The bottles of tablets should be stored away from direct light and heat. Media suitable for culture of both bacteria and micro-fungi can be obtained from Oxoid Ltd, Southwark Bridge Road, London, S.E.1, who also publish a useful manual giving details of techniques.

4. Preparation and sterilization of media

a. Tablets not containing agar. 'Broth' tablets are self-mixing. Add 10 cm^3 of distilled water to one tablet in a test-tube or bottle and heat-sterilize.

b. Tablets containing agar. For the preparation of plates, 10 cm^3 of distilled water are added to two tablets, which should then be allowed to soak for fifteen minutes before being heat sterilized. This amount is sufficient for one plate. The bottles of media supplied by Oxoid Ltd (see above), usually contain 100 tablets which is therefore sufficient for fifty plates (for slopes, 1 tablet in 5 cm^3 of distilled water is sufficient).

It should be noted that if a fluid inoculum is to be added, the volume of such an addition should be subtracted from the volume of distilled water required for the reconstitution of the medium. In this way the concentration of the ingredients in the formula will not be lowered and the gel strength of the agar will not be weakened.

For some of the practicals in which the class are required to make their own pour plates, 1 oz wide-mouthed screwcap McCartney bottles are more convenient than test-tubes for sterilizing processes. After sterilization, they can be kept in a water bath at about 45°C. At this temperature the agar will remain liquid and ready for making a pour plate (see below) and will also be at a temperature which will not kill any inoculum to be added.

If the tubes are used, they should be supported in racks in the autoclave and in the water bath.

If screw-topped bottles, such as McCartney bottles, are used, the screw caps should not be done up tightly. Tubes should not contain more than half their volume of medium, otherwise it may boil over and wet the cottonwool plugs. A spare tube of medium should always be included for use as a control later.

If a pressure cooker is used, a small amount of water is put in the bottom and with the lid firmly 'fixed' and valve open before it is heated. The valve is closed when there is a strong jet of steam, and the pressure watched until it rises to 15 lb/in^2. (Most domestic pressure cookers do not, in fact, have a pressure gauge.) The heat is then turned off and only when the pressure has returned to zero is the valve opened, so that a vacuum is not created. If time allows the tubes and bottles should be left in the pressure cooker or autoclave until they are cold. After removal the plugs on the tubes should be covered with paper or foil and the caps on the bottles screwed down, to prevent contamination, unless they are to be used at once.

5. Plating out the medium

Before being poured onto the plates, the medium must be melted by heating the bottles or tubes in a water bath. Heat the agar to 100°C. If hot medium is immediately poured into a Petri dish condensation occurs inside the lid. It is best therefore to allow the media to cool down to just above 40°C, before plating out. Even at 40°C condensation can occur, so that it is best first to warm the Petri dishes slightly. The actual process of plating out can be a critical moment for the entry of contaminants and should, therefore, be carried out away from draughts and open windows. After pouring the liquid medium onto the plate, replace the lid as quickly as possible and rotate the plate to ensure even distribution. Media with an agar base gel at 38°C.

If the possibility of contamination is suspected, it is as well to plate out the medium a few days before it is required, so that any plates showing contaminants can be discarded.

6. Inoculation of plates

For this course 'streak plates' are mostly used. An *inoculating loop* is the best form of inoculating instrument, and is made from a loop of wire, 2–3 mm in diameter, inserted in a wooden handle. Nichrome wire (0·5 mm diameter) is the most suitable.

Hold the nichrome loop in a Bunsen flame until red hot to sterilize it and then let it cool before dipping it in the inoculum. Raise the lid of the Petri dish to allow quick streaking across the plate as in text figure 16. Then replace the lid. A caution should be given here – to avoid digging into the agar, the inoculating loop should be held nearly horizontal while streaking the plate and stroked *lightly* across.

7. Labelling

All plates and tubes should be labelled immediately after inoculation and before being incubated. A Chinagraph pencil is best for this.

8. Conditions for growth and methods of inhibiting growth

With the aid of an incubator, which can be controlled at a fairly low temperature, cultures of bacteria can be raised to the right stage with a fair degree of precision in timing. This is obviously important when fitting in with a school timetable.

At 37°C bacterial colonies will form within twenty-four to forty-eight hours while moulds tend to develop best at 25°C (or room temperature). Moulds will form on plates if they are left for more than two days in an incubation temperature of 37°C. If bacterial colonies are to be observed, unobscured by a fungal mycelium, it is best to place the plates in a refrigerator after thirty-six hours or so in the incubator. This will inhibit growth which will begin again if incubation is resumed. Alternatively growth can be totally inhibited by placing a circle of filter paper, on which a few drops of 40 per cent formalin have been pipetted, inside the lid of the Petri dish. The dish should then be inverted, with the lid and filter paper undermost.

9. Disposal of cultures and cleansing of utensils

A container of 2–3 per cent Lysol, or another similar disinfectant, should be provided for the reception of all dishes and tubes when they are no longer required. Solid media can either be wrapped in newspaper and burned or re-sterilize and while still hot flush down the sink with plenty of hot water to clean the pipes. All glassware should be washed in very hot water to which disinfectant has been added, and then thoroughly rinsed in cold water. Detergents and strong disinfectants are toxic to micro-organisms and all traces must therefore be removed by thorough rinsing before the glassware is used again. Disposable Petri dishes can be autoclaved to destroy them.

Labelling and recording

Experiments with micro-organisms are inevitably rather 'complicated' in the sense that several containers of different materials may be in use at the same time. This underlines the importance of proper labelling *immediately* a particular experiment has been set up. Failure will almost certainly follow if this is not done. Chinagraph wax pencils write well on glass, while the various kinds of fluid marker (Magic marker, etc.) are also good. All can easily be removed afterwards with a solvent such as benzene.

Similar warnings to those above apply with, perhaps, even greater emphasis, to the recording of results, which must be obtained systematically, and written down at once. It is wise to discourage the use of loose pieces of paper for making records in the laboratory as these easily get lost and the information thereafter may well be unobtainable. One answer which works well is to insist that all students have a small, rough notebook (the size that will fit easily into a pocket) in which records are kept temporarily for transfer to a more permanent place later on, when the experiment is completed and written up.

A home-made incubator

A simple incubator may be constructed from readily available materials which, despite its simplicity, will provide the requirements for all but the most critical bacteriological techniques.

A box with overall dimensions of about 18 × 12 × 12 inches is the basis for construction. The source of heat is two electric light bulbs, and a thermostat of the type manufactured for use in tropical fish tanks provides the means of controlling the temperature within the incubator (see figure G1).

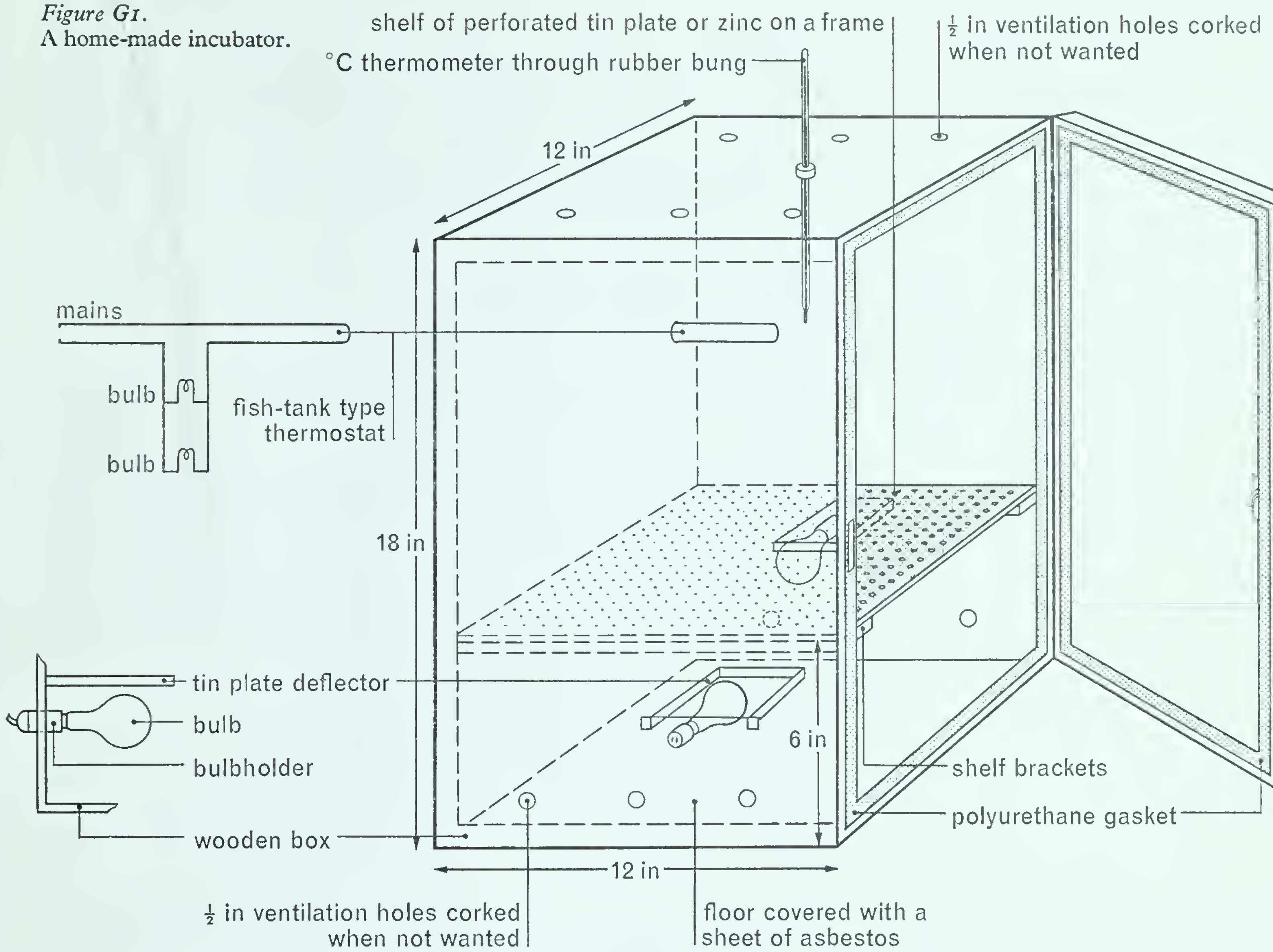
The shelf is large enough to hold up to twenty 4 inch Petri dishes and the bulbs are placed beneath a baffle to prevent overheating of the shelf.

Although a 40 W lamp will probably be adequate to maintain a constant temperature, the warming up period is rather prolonged and for this reason a second 60 W lamp is advisable.

The thermostat, obtainable from any shop dealing with tropical fish is capable of maintaining the air temperature within the incubator between the limits of ± 1°C of the required temperature, while the contents of a Petri dish should not vary by more than 0·2°C. Lagging with Polyurethane increases the efficiency of the incubator.

At least two shelves should be made from perforated steel and some provision for removal and height adjustment would be a help if culturing in large flasks is to be attempted. Small wooden slats screwed to the walls at intervals of two inches provide an easy means of adjusting the shelves.

Figure G1.
A home-made incubator.


shelf of perforated tin plate or zinc on a frame
½ in ventilation holes corked when not wanted
°C thermometer through rubber bung
12 in
mains
bulb
bulb
fish-tank type thermostat
18 in
tin plate deflector
bulb
bulbholder
wooden box
6 in
shelf brackets
polyurethane gasket
12 in
½ in ventilation holes corked when not wanted
floor covered with a sheet of asbestos

2.12
Growing bacteria from the air
Notes on teaching

Apparatus required per group of four
4 Petri dishes containing sterile Blood Agar Base
A piece of black paper

The investigation itself requires little comment as full details are given in the text. In class, this provides an opportunity for further discussion of Pasteur's work with particular reference to his studies of bacteria carried in the air for instance – in connection with his experiments to disprove the theory of spontaneous generation.

As in all these experiments, the necessity for a control experiment should be emphasized. This is a point which might well be discussed further in class.

For this experiment the class could work in groups of four and to save Petri dishes, one control to a class is sufficient. Blood Agar Base is obtainable from Oxoid Ltd (see above).

Bacterial colonies will be apparent after incubation for 24 to 48 hours at 37°C. If it is not possible to examine the cultures in class at this stage, further growth can be inhibited by removing the cultures from the incubator and placing a circle of filter paper, on which a few drops of 40 per cent formalin have been pipetted, inside the lid of each Petri dish. The dish should then remain inverted, with the lid and filter paper undermost.

Growing bacteria at different temperatures
Optional practical

If time permitted, a useful optional practical would be to try growing bacteria at different temperatures, not only at 37°C, and to see what happens. *How much* does an increase or decrease in temperature alter the rate of growth of bacteria? How are we going to measure the rate of growth? These are important points for discussion. Various possible conditions are a refrigerator, room temperature, on a radiator, out of doors, and so forth.

2.13
Bacteria on ourselves

Notes on teaching

Apparatus required per pair
3 sterile Petri dishes containing sterile Blood Agar Base
A piece of black paper

This can be a very good experiment and the moral to be drawn from it is obvious enough! The results obtained by the class could form the basis for a general discussion on cleanliness. In these class practicals it is important, whenever possible, to summarize the results obtained by the whole class on the board, particularly if, as usually happens, they are somewhat conflicting. Children at this age should realize that scientific discoveries are made not just on the basis of one experiment, but on many, sometimes conducted independently by a number of different people.

As in the previous experiment the number of Petri dishes can be reduced by using a single control for the class. Blood Agar Base is obtainable from Oxoid Ltd (see above).

We have been concerned in this experiment only with bacteria from our hands. What about those from other places? There is a wide range of possibilities here for further investigation.

Finger print after drying hands on a dirty towel, a paper towel, and in hot air
Optional practical

A Petri dish is plated out with Blood Agar Base and inverted. The base is divided into three sections labelled A, B, and C, by using a Chinagraph pencil. Three people, A, B, and C dry their hands in different ways. A on a communal and presumably fairly dirty towel, B on a disposable paper towel, and C in hot air over a low Bunsen burner. Each makes a light finger print in the appropriate segment of the Petri dish which is then incubated at 37°C for two days. Bacterial colonies can then be compared.

2.2
Are there bacteria in milk?

Apparatus required per pair
2 sterile Petri dishes plated with sterile MacConkey Agar.
1 nichrome inoculating loop.
1 tube of *either* raw milk diluted to 1/1,000
or pasteurized milk diluted to 1/100

Notes on teaching

The study of milk and the action of micro-organisms upon it, and also the benefits we enjoy as a result of some of these activities, provide an absorbing subject with almost unlimited ramifications. Viewed in the present context, it also gives another good opportunity for stressing the usefulness of biology to man. A visit to a milk collecting centre would afford an opportunity not only of seeing general hygienic principles put into practice, but also some of the tests made on milk.

The action of many organisms in milk is highly beneficial to us. For instance, cheeses such as Gorgonzola or Danish Blue, depend for their colour and characteristic taste on the action of particular strains of the fungus, *Penicillium.* Other micro-organisms are less beneficial such as the bacteria which cause milk to go sour when we wish it to remain fresh and fit to drink. It is with these that we are concerned here.

Streak plates are easy to prepare and full instructions are included in the text. There is little that can go wrong. As a postscript to this section it might be well to consider the basic principles underlying the commercial control of bacteria in milk. These are:

Immediate cooling after milking, which restricts the growth of the bacteria invariably present.

Pasteurization – this does *not* sterilize the milk but destroys most of those bacteria which do not form spores and which are always present. Provided a suitable temperature is maintained for a sufficient duration of time, most heat-resistant pathogens are also killed, e.g. the tubercle bacillus.

Raw milk is usually obtainable from dairies on order. If raw milk is used, it should be kept for twenty-four hours before use in order to ensure a good growth of organisms. Pasteurized milk should be kept for forty-eight hours before use.

This experiment is merely to show the presence of bacteria in milk. Hence stale milk is used in order to show results. MacConkey Agar, obtainable from Messrs Oxo Ltd (see above), selects coliforms, but the number of colonies cannot be taken as an indication of the total bacterial population.

If bacteria grow in the culture made from pasteurized milk this cannot be taken as an indication either (*a*) that the milk has been improperly pasteurized, or (*b*) of the numbers of bacteria present in the original sample. Pasteurization does not destroy all bacteria but only those of pathogenic importance.

If B has grown any bacteria, can this be due to improper sterilization or to contaminants after sterilizing?

Streak plates

Streak plates are made by inoculating the surface of a solid-nutrient medium, either with a swab of inoculum or with a nichrome wire loop dipped in the inoculum. The streaking should be done so that the second set of streaks intersects with the first set of streaks as in figure 16. The colonies should then be distinct, and should be close together, or even confluent, at the points of intersection of the two sets of streaks although certain kinds of bacteria, notably *Streptococci*, will never produce confluent colonies.

Phosphatase test for pasteurization

On a commercial scale, the most effective way of testing the success of pasteurization in killing the tubercle bacillus is as follows. Raw milk invariably contains the enzyme phosphatase which is slightly more heat-resistant than the tubercle bacillus itself. If pasteurization has been properly carried out, the enzyme should therefore be destroyed. In the test, a small quantity of milk is added to a solution of disodium phenylphosphate. If active phosphatase is still present, the reagent is split up liberating phenol, which then reacts with an indicator to give a blue colour. The degree of blueness shows the amount of enzyme still present.

Film Loop

A Film Loop entitled 'Control of bacteria in food', NBP–67 shows how, under suitable conditions bacteria can multiply rapidly. Milk samples, treated in different ways, are tested for bacteria and show that only a few bacteria survive pasteurization. Cooling milk is also a method of slowing down growth of bacteria. Although ice-cream is a potential source of bacterial infection, modern methods employed in its manufacture involve the elimination of all pathogens.

2.21
Pasteur discovers why wine can go sour

Notes on teaching

The standards insisted upon in the production of milk aim at (*a*) the reduction in the number of contaminating organisms entering the milk from all sources during and after milking, and (*b*) the prevention of increase in the number of organisms already present, during the period following milking. Such measures include cooling, keeping churns cool, and pasteurization. All these measures are designed to take into account the physiological characteristics of the micro-organisms, the chief of which is the ability to increase in numbers at certain optimum temperatures.

2.22
Can we improve the keeping qualities of milk by pasteurization?

Apparatus required

per class	*per group of four or six*
Water bath at 63°C	6×1 oz McCartney bottles Raw milk (or pasteurized milk forty-eight hours old) 6 boiling tubes

This experiment involves the maintenance of a fairly exact water bath temperature of 63°C and holding this temperature for thirty minutes. It is worth while noting that at the normal air temperatures experienced during the late autumn and winter pasteurized milk should keep for at least three days while raw milk may turn sour in thirty-six hours. However, after two or three days, the milk samples should not be tested for sourness by tasting, since there may be a considerable culture of bacteria present, especially in the raw milk. If raw milk is not available the experiment could be used to compare fresh pasteurized milk with pasteurized milk that is forty-eight hours old.

For pasteurizing the raw milk samples it is best to use 1 oz McCartney bottles (28 cm^3), which have screw caps and rubber cap linings. Hence they are completely watertight.

The McCartney bottles should be *completely* immersed in the water bath.

The 'clot on boil' test is still used by milk factories but is rather slow since it depends upon the incubation of the milk samples for twenty-four hours before the test. Similarly the methylene blue test, also used in milk testing, depends upon incubation.

2.23
Testing milk for freshness (The resazurin test)

Apparatus required

Samples of raw, laboratory pasteurized and commercially pasteurized milk
Resazurin tablets
4 test-tubes and rubber bungs
Water bath

For this test, which will probably be done as a demonstration, it will be best to make a fresh sample of laboratory pasteurized milk and also to use fresh samples of raw and of commercially pasteurized milk, since different degrees of colour in the resazurin are wanted.

The method uses the dye resazurin (obtained in tablet form, in bottles of 10 or 100, from Messrs Hopkin and Williams Ltd, Freshwater Road, Chadwell Heath, Essex) which causes the milk, upon reduction, to change from blue through pink to white. The reactions concerned in the test are:

$$\underset{\text{blue}}{\text{resazurin}} \rightarrow \underset{\text{pink}}{\text{resorufin}} \rightarrow \underset{\text{white}}{\text{hydroresorufin}}$$

Resazurin really tests the metabolic activity of the bacteria present rather than the number or kind of bacteria. Nevertheless, those kinds of bacteria which cause souring of milk (the lactic acid bacteria and the coliforms) are those causing the change of colour in the resazurin. Again, certain types, although present, cannot be detected by a dye reduction test. However, the bacterial flora of milk is sufficiently typical to permit use of this test as a means of estimating bacterial content. Tubes decolourized to white or pink or white mottling have failed the test.

It should be noted that resazurin is very sensitive to light. The solution and the milk sample should, therefore, be protected throughout from bright sunlight.

On completion of the demonstration, the tubes can be removed from the water bath, enclosed in paper covers to exclude the light, and suspended in clamps or put in a test-tube rack. The further colour changes which take place as the milk sours can be noted after one and two days.

Quantitative analysis of a milk sample by plate count method

A more accurate method of estimating the bacterial content of a milk sample is by making a plate count using MacConkey Agar which selects coliforms, but is not a method of making a total bacterial count.

1. Draw up a 1 cm^3 of milk sample in a sterile 1 cm^3 pipette, plugged with flamed cottonwool at the mouth end, and add to 9 cm^3 autoclaved tap water. Use fresh pipette for each dilution.
2. Mix ten times by blowing in and out of pipette gently. Draw up 1 cm^3 and transfer to second 9 cm^3 giving a dilution of 1/100.
3. Repeat twice more to obtain dilutions of 1/1,000 and 1/10,000.
4. Starting with the highest dilution–(1/10,000), withdraw some fluid with a 50-dropper pipette (see below). First test dropping action back into the bottle and then transfer 1 drop to a labelled segment of a MacConkey Agar plate, which should be *dry*.

(Prepare the day before and leave overnight in an incubator at 37°C with the lid on.)
5. Using the same pipette, withdraw sample from 1/1,000 solution and place on another labelled segment of the plate.
6. Repeat for the 1/100 sample.
7. Incubate, lid side up, at room temperature for four days or at 37°C for one or two days; invert plate only when the drops are dry. Coliforms will show in twenty-four hours if incubated at 37°C.

MacConkey Agar will select coliforms only, and hence a comparison can be made between the number of coliforms in raw milk, laboratory pasteurized, and commercially pasteurized samples. If milk agar is used, a more complete count of organisms is possible but they will not show up so well.

The greater the dilution, the more separated the clumps of organisms become. An optimum count of between twenty and thirty colonies is to be aimed at.

Calculation of the number of colonies per 1 cm^3 of original sample is made as follows:

Number of colonies counted $\times 50 \times 100 =$ Number of colonies of the original sample.

The count is repeated for the 1/1,000 and 1/10,000 dilutions.

Theoretically they should give the same number of colonies per 1 cm^3 of the original sample (this figure should also represent the number of coliforms per 1 cm^3 of the original sample). In practice the plate count nearest to thirty colonies is usually selected. Replicated drops rarely give identical counts. The question therefore arises of random sampling.

Under the most favourable conditions a specimen of raw milk may contain a total bacterial population (all kinds) of as little as 500 bacteria per cm^3; but under bad conditions the numbers may reach even several million per cm^3. The standards given below indicate the degree of bacterial contamination allowable in the case of designated milks:

'Certified' milk must contain not more than 30,000 bacteria per cm^3 and no coliform bacillus in 0·01 cm^3.

'Pasteurized' milk must contain no coliform bacillus in 0·01 cm^3 and must satisfy the phosphatase test.

'Tuberculin Tested' milk must contain not more than 200,000 bacteria per cm^3 and no coliform bacillus in 0·01 cm^3.

Making a 50-dropper

1 drop $=0 \cdot 02$ cm^3. It may be of interest to know how to make a pipette which can deliver 50 drops per cm^3.
1. Using soda glass tubing 7–8 mm diameter, heat, pull out, and break.
2. Run the pipette into the hole in a metal gauge. This is a metal plate through which there is a hole of 0·95 mm diameter, obtainable from Messrs R. B. Turner & Co. Ltd, Inocula House, Church Lane, E. Finchley, London, N.2. Mark the tube with a Chinagraph pencil at the right gauge.

3. With a diamond cutter score the tube at this point and break.
4. Flame the end of the pipette until a sodium-yellow is obtained. This does not melt the glass but burns the grease of the Chinagraph pencil.
5. Plug the large end of the pipette with cottonwool.
6. Make a number of such pipettes and place them in a 1 inch tube in which cottonwool is placed at the bottom. Plug the tube with cottonwool. Dry sterilize in an oven at 160°C for 2 hours, or autoclave at 15 lb/in^2 for 15 minutes. Dry off slowly in the oven.

Film Loop for 800E projector

An excellent Film Loop entitled 'Practical demonstration of Koch's postulate', Number BM/259, with a running time of 3 minutes, is obtainable from Gateway Educational Films Ltd, 470 Green Lanes, London, N.13, or from Technicolor Ltd, Bath Road, Harmondsworth, West Drayton, Middlesex. The film illustrates how pure cultures are made as well as the methods of making streak plates and of streaking an agar slope.

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Chapter 3

Bacteria and health

Objectives

Much has already been said about the growth of bacteria and how they can cause diseases of one kind or another. The intention of this chapter is to show the link between modern methods of hygiene and the maintenance of health. The experimental work is concerned with the use of disinfectants and killing bacteria.

The sequence of development of the chapter is as follows:

1. Under optimum conditions, bacteria multiply at a rapid rate.

2. Bacteria are present in air in the highest concentrations where human overcrowding results in a high air humidity.

3. The bacterial population of the air can be tested by means of a slit sampler.

4. Water contains bacteria and therefore water supplies have to be constantly tested and purified.

5. Bacteria can cause decomposition of food, but correct handling and preservation of food can prevent contamination.

6. Bacteria in the air can cause suppuration of wounds. The use of antiseptics reduced the number of deaths after surgical operations.

7. Bacteria are capable of growing in certain disinfectants such as phenol, when much diluted.

Suggestions for practical work

	Section		Page *Text*	*Guide*
Class practical work	3.2	Bacteria and disinfectants	35	31
Optional practical work	3.21	Comparing the action of different disinfectants on soil bacteria	36	32
	3.21	The effect of different strengths of disinfectant on a pure culture of bacteria	36	32

3.1
Bacteria in millions

Notes on teaching

We are not concerned at this stage with the actual mechanism by which micro-organisms multiply, but with their ability to do so quickly. When the results, in terms of numbers, are calculated, the question will almost certainly arise, 'What checks their increase?' Two reasons have been given, but there are others which might be enlarged upon, such as the production by the bacteria themselves of toxic substances, as well as the existence of viruses which can destroy bacteria (bacteriophages). The old adage of ‘little fleas have lesser fleas upon their backs to bite ’em’ is always an interesting speculation!

Doetsch (1960) gives the weight of bacteria as they multiply, ‘. . . that a single rod will weigh 0·00000000571 mg or that 636 billion bacteria would weigh 1 g, or 636,000 billion a kg: after twenty-four hours the weight of the bacteria amounts to about 1/40 mg; after forty-eight hours nearly 1 lb; after three days it approximates to a weight of 7·5 million kg or 148,356 hundredweight.’ Although this gives an idea of the potential ability of bacteria to increase, the limiting factors should be stressed.

3.11
Clean air
Notes on teaching

Pasteur showed that bacteria, yeasts, and fungus spores are normally present in the air. Micro-organisms can be carried in the air in several ways – in air currents, on dust particles or, most frequently, on droplets of moisture. Thus a rise in the humidity of the air is likely to increase its bacterial population, although air temperatures must also be taken into account. Humidity rises rapidly in a poorly ventilated room in which there are a lot of people. A mention of the injurious effects of fog, smog, and dust in the air could lead to discussion of the means by which germs are transmitted by droplet infection from one person to another (coughing, sneezing, dirty handkerchiefs, and so forth). In this connection it is interesting to note that the risk of infection by ‘droplets’ varies inversely as the cube of the distance from the source of the infection. Since the risk becomes much greater where there are a number of people in a confined space, the Factory Acts stipulate that there shall be 400 cu ft of air space per person. The spacing of beds in hospital wards, desks in classrooms, and stalls in cattle sheds and stables is very important. Rain, snow, and hail help to remove large numbers of micro-organisms from the air. Thus water reaching the ground by precipitation is not sterile.

Aerosol sprays (delivering droplets 5–120 μ diameter) contain germicides sufficient to kill most organisms with which the droplet collides.

The slit-sampler method of estimating the population of micro-organisms in a sample of air depends upon a known volume of air being drawn over a nutrient medium in a Petri dish. But this method fails to detect those organisms which are unable to grow under the particular conditions used. Slow-growing forms are also readily overgrown by more vigorous ones.

Slit-samplers are used to test the air of factories where there is likely to be a high degree of air pollution by either dust or micro-organisms. The extent to which the slit-sampler is able to test the number of micro-organisms in a part of a room or in a whole room is a question that might be related to the problem of sampling.

3.12
Clean water
Notes on teaching

The idea of contaminated well water can be linked with the work on soil bacteria (section 1.22) and the question of what soil water contains. The whole problem of sewage disposal and river pollution may arise and what effect it has on fish and other river and estuarine fauna.

Alternative experiment – Testing water for coliforms

Samples of drinking water, pond water, and distilled water are tested for coliforms, using boiled water as a control.

For this test MacConkey Agar (obtainable from Oxoid Ltd, see above), is used as the nutrient medium. It contains lactose which is fermented by any coliforms present in the water sample, the fermenters appearing as pink or red colonies, the non-lactose fermenters being colourless.

For each sample to be tested, dissolve two tablets of MacConkey Agar slowly in 9 cm^3 distilled water and sterilize by autoclaving for 15 minutes at 15 lb/in^2. After cooling to 45°C, 1 cm^3 of the water sample is added and a pour plate made in a sterilized Petri dish. The dishes are then incubated for twenty-four hours at 37°C.

A good point for discussion might arise if the drinking water from which the sample is taken is heavily chlorinated, with the result that only a few colonies of coliforms develop or none at all.

Test for coliforms in water

The standard test employed by analysts to determine the presence of coliforms is in three parts:

a. The presumptive test

This test gives an indication that coliforms are present.

Graduated amounts of water are placed in fermentation tubes of lactose broth, each containing at least twice as much medium as the sample to be tested. Five such tubes are inoculated with 100 cm^3 samples of water to be tested and incubated at 35°C for forty-eight hours. If gas is produced then coliforms are presumed to be present since they are practically the only bacteria capable of fermenting lactose with the production of gas. The test determines the most probable number (MPN) of coliforms per 100 cm^3 sample.

There are, however, other bacteria capable of fermenting lactose with the formation of gas (*Clostridium* and *Aerobacter*). Therefore a confirmatory test is needed.

b. The confirmed test

Samples from tubes of positive presumptive tests are transferred to a selective medium such as eosin methylene blue (EMB) agar: and streak plates are made to obtain isolated colonies. After incubation at 35°C for twenty-four hours, the plates are examined and if coliforms are observed the test is recorded as positive.

c. The completed test

This test establishes that typical colonies apparent on the plates are of the coliform group, by inoculating a lactose fermentation tube with isolated colonies, and examining these under a microscope.

The chlorinated water of most cities should reveal no coliforms of any kind in 100 cm^3 samples, otherwise treatment should be considered inefficient. For non-chlorinated water the standards required vary with different types of water but, in general, where there are more than 1 to 5 coliforms per 100 cm^3 sample it is regarded as unfit for drinking.

Reservoirs

Damming the exit of a natural lake, or of a valley in rocky districts, restricts the flow of algae which tend to clog the filters. This may become a pressing problem with London reservoirs which draw their water from slow-flowing, highly polluted sources. Here the reservoirs are of the earth-bank type which also encourage algal growth. This can be restricted either by causing the water to circulate or by adding crystals of copper sulphate.

3.13
Clean food

Notes on teaching

It follows that whatever is food for ourselves or for other animals is also food for micro-organisms. Decomposition ensues after the death of the animal, or after harvesting vegetables and fruit. Of course enzymes existing in the food will cause part of the decay but micro-organisms, whether originally present, or added during handling, are chiefly responsible for the decomposition which will proceed at different rates according to the temperature.

The two points we are trying to emphasize are the clean handling of food to prevent contamination (see section 2.13), and an understanding of the effects of temperature on the rate of decomposition. The experiment on milk (see section 2.23) showed that at room temperature souring is fairly rapid, but that if milk is kept in a refrigerator it remains fresh longer.

Such principles are, of course, evident in a great many modern methods of food production and packaging. Probably the class will have plenty of examples to offer. The problem raised by the correct handling of deep-frozen foods comes to mind. For instance the withdrawal of poultry from deep-frozen units for display on the counter involves a partial thawing out. If they remain unsold, they may be returned at the end of the day to the deep freeze, to be taken out once more the following day. Do such conditions encourage decay? If refrigeration destroys the smell of a sour broth when it is first removed from the refrigerator, how can we know that it is sour? There are obviously a whole host of questions of this kind that could be usefully discussed. (See the alternative experiment at the end of this section of the guide.) It is interesting to note that, even at very low temperatures some forms of bacteria can survive and multiply; thus the temperature of a deep freeze should never rise above −15°C and is usually efficient for the storage of most foods at −18°C.

Film Loops

a. An 8 mm Film Loop, entitled 'The problem of feeding (2): the house fly as a sucking insect', NBP–7, illustrates admirably the various ways in which house flies can spread disease. It shows the suctorial method of feeding and the movements of the legs during the cleaning of the hairy body of the insect – both ways in which bacteria can be transferred by flies to food.

b. The 8 mm Film Loop 'Control of bacteria in food', NBP–67, described in section 2.2, also illustrates this section.

Alternative experiment – Does refrigeration destroy bacteria?

Two tablets of Nutrient Broth No. 2 (obtainable from Oxoid Ltd, see above), are dissolved in 20 cm^3 distilled water in a flask or test tube and allowed to go sour. Does the broth now smell sour? Place the broth in an ordinary domestic refrigerator for twenty-four hours. After removing it from the refrigerator, does the broth still smell sour? Examination of a drop of the broth under the high power of a microscope will no doubt reveal large numbers of bacteria.

3.2

Bacteria and disinfectants

Notes on teaching

The whole subject of sepsis and antiseptics is one which could easily involve the teacher in complicated explanations of such things as antigens and antibodies. The important issue here is that disinfectants are one of the ways of preventing the entry of germs into the body.

It should be noted that *disinfection* means the destruction of disease-producing micro-organisms, while the term *sterilization* is reserved to denote the complete eradication of all micro-organisms from the matter being sterilized. There then remains the explanation of *antisepsis* which is really the use of certain substances to prevent septic conditions developing in the body.

It is strange that the work of Redi and Spallanzani failed to suggest any connection between microbes in the air and the mortality resulting from surgical operations and it was not until 1843 that the American anatomist and poet, Oliver Wendell Holmes, urged improved conditions for maternity cases. This was followed a few years later, by the Austrian doctor, Semmelweiss, who showed that by making his medical students wash their hands in antiseptic on leaving the dissecting rooms, before entering the maternity wards, he could reduce the number of deaths from sepsis.

Another twenty years was to go by, however, before Lister introduced, in 1867, his theory that sepsis in wounds is caused by micro-organisms.

Lister was concerned with the contamination of wounds and the exclusion of germs from the air around the wound as well as from the wound itself. From the simple methods Lister employed, the whole science of asepsis has arisen, which now forms the foundation upon which modern hospital procedure is based.

A brief description of modern operating methods may help to establish a link with some of the practical work undertaken. The term 'scrubbed up' used by theatre staff means that the walls and floor of the theatre are hosed down with disinfectant before an operation and that all dressings, clothing worn in the theatre, and instruments have to be sterilized under pressure, for a certain length of time. Germs are present on the body and on clothing but more so on the latter than on washed and scrubbed skin. Thus staff and surgeons wear the minimum of clothing under their operating gowns. A mask over nose and mouth, a cap tied over the hair, and rubber gloves complete the cover-up of the body.

Comparison of an operating theatre in Lister's day with the picture of a modern theatre illustrates the many advances made in surgery.

Joseph Lister (1827–1912)

Lister was born in Essex of Quaker parents, but after qualifying at University College, London, he spent much of his working life in Scotland, first in Edinburgh and then as Professor of Surgery at Glasgow. In 1867 he returned to Edinburgh where he became Professor of Clinical Surgery. Thus, much of his life was spent in teaching and he numbered among his pupils many medical men who later became famous.

Lister was best known for his part in developing methods of antiseptic surgery and in 1857 he wrote a classic monograph entitled *An essay on the early stages of inflammation.* In 1865 he read of Pasteur's work on putrefaction and fermentation which led him to argue that such processes might be similar to conditions found in septic wounds.

In the same year Lister began using lint dressings saturated with carbolic acid and later developed the idea of a spray of carbolic playing on the wound during an operation. It is interesting that although it was already known that coal tar was a useful germicide, Lister chose carbolic acid as a disinfectant when he learned that in Carlisle it was used successfully to rid garbage of odour. Later, when it became apparent that his crude carbolic caused secondary lesions, he started using a more dilute solution.

Lister was well known for his work on antiseptic surgery, and yet in 1873, he discovered *Streptococcus lactis*, the organism responsible for the souring of milk, and later he perfected methods for obtaining a pure culture. He extended the idea of fermentation to include the surgeon's problem of the putrefaction of wounds, but he was interested in proving that micro-organisms are the cause and not the result of ferments.

Film strip

A film strip, Number CGA/608, produced by Common Ground, 44 Fulham Road, S.W.3, entitled 'Lister and Antiseptics' describes his discoveries.

3.21
What strength of disinfectant kills bacteria?

Apparatus required per group of four or six
7 test-tubes $5\times\frac{3}{4}$ inches and cottonwool plugs
20 cm^3 0·4 per cent phenol solution
70 cm^3 soil inoculum
0·004 per cent Brom-cresol-purple in a dropping bottle
Distilled water in a conical flask
Small glass funnel or 10 cm^3 disposable syringe with needle-holder removed.

Notes on teaching

Many disinfectants in common use, such as Lysol, Jeyes's Fluid, Carbolic acid, Cresol, and Sudol, are tar derivatives containing phenol, which is present in most of them at a strength of about 50 per cent. These disinfectants are listed as 'black fluids' in the Pharmacopœia.

The question to be solved is at what strength a particular disinfectant will destroy the bacteria present. Thus the experiment involves making a series of dilutions of the disinfectant. Most of the commercial disinfectants mentioned above, turn milky in solution. Thus it is probably best to use pure phenol, although Lysol is a possible alternative (see optional practicals below).

Add the same amount of inoculum to each dilution. But this may be the cause of varying results, since if soil is used as a ready source of bacteria, we are immediately faced with two unknowns: (*a*) The number of bacteria in each inoculum will not be constant, and (*b*) there will not necessarily be the same kinds of bacteria in each inoculum, and some may react differently from others to the disinfectant. The two variables may account for different results being obtained in the replicates. However, some assurance of uniformity, under these circumstances, can be achieved by (*a*) using soil specimens from the same samples of soil for each of the soil solutions, and (*b*) by mixing the soil solution very thoroughly before filtering. These are questions which might well be considered in class discussion and related to the problem of sampling as a whole. Avoid using clay soil because it produces a cloudy filtrate.

This is an interesting experiment since it demonstrates the killing of bacteria above a certain concentration of Lysol (phenol) and the ability of bacteria to feed upon the carbon present in phenol at a certain dilution, while below that dilution there is insufficient carbon to act as food for the bacteria. It will be interesting to see if these are the interpretations put on the results by the class.

It is clearly a very open-ended experiment, since a variety of results may be obtained for which a number of reasons could be given. For instance the turbidity in replicated sets may not occur at the same dilution in each, or there may be turbidity throughout several of the dilutions down to the last one. Does this relate, in any way, to the question of sampling? Can it be proved by using different soils? Again why is distilled and not tap water used?

It is interesting to note that quite recently certain bacteria have been found to be capable of feeding under certain conditions, upon mercury compounds and other unlikely substances, thus rendering such compounds unsuitable for use as germicides.

Above a strength of 0·1 per cent phenol, bacterial growth is inhibited, whilst at approximately 0·02 per cent phenol can be used as a substrate producing good bacterial growth, indicated by turbidity. The bacteria cause a lowering of pH due to the accumulation of CO_2 and possibly also to the production of organic acids. This can be shown by the use of an indicator. Thus after results of turbidity have been recorded the presence (or absence) of bacteria can be verified by culturing samples for each of the tubes in a series. The use of the indicator is another means of verification.

Procedure

1. Test-tubes. The test-tubes should be marked at the 10 cm³ and 20 cm³ levels with a Chinagraph pencil.

It is suggested in the text that the pupils should use a glass funnel to assist them in making the dilutions of disinfectant and in adding the inoculum. If this proves difficult, try a 10 cm^3 disposable syringe (without needle holder).

2. *Soil inoculum.* It will be best to make up beforehand, sufficient soil inoculum to supply the whole class.

Grind up 25 g soil in a pestle and mortar and add it to 100 cm^3 distilled water, leave to stand for fifteen minutes, and then filter the supernatant fluid.

3. *Ammonium sulphate-potassium acid phosphate buffer mixture.*

Since this experiment demonstrates acid production arising from the growth of micro-organisms in the soil, one should aim at a neutral solution to begin with. The following buffer mixtures give the range of pH indicated and, if the pH of the soil inoculum obtained in (*2*) is not tested, it will probably be best to use (*b*) below with a neutral pH.

a.	Ammonium sulphate	0·2 g	+ 5 g dextrose
	Potassium dihydrogen phosphate	0·2 g	
	Distilled water	to 100 cm^3	
	pH 4·7		

b.	Ammonium sulphate	0·2 g	+ 5 g dextrose
	Potassium dihydrogen phosphate	0·1 g	
	Dipotassium hydrogen phosphate	0·1 g	
	Distilled water	to 100 cm^3	
	pH 6·8		

c.	Ammonium sulphate	0·2 g	+ 5 g dextrose
	Dipotassium hydrogen phosphate	0·2 g	
	Distilled water	to 100 cm^3	
	pH 7·9		

To the filtrate obtained in (*b*) above, add an equal volume of (*c*).

4. *Phenol dilutions.* 0·4 per cent solution of phenol is made by dissolving 4 g of pure phenol in 1 litre of distilled water.

Provide 20 cm^3 0·4 per cent phenol as the strongest solution to be placed in Tube 1. The test-tubes should be $5\times\frac{3}{4}$ inch. Make a series of five other dilutions in the rest of the Tubes 2 to 6 each containing 10 cm^3 diluted phenol. A seventh tube, the control, is added to the series and contains 10 cm^3 distilled water only. By adding 10 cm^3 of the inoculum and buffer mixture to 10 cm^3 0·4 per cent phenol in Tube 1, an initial strength of 0·2 per cent phenol is obtained. Subsequent dilutions in Tubes 2, 3, 4, 5, and 6 will be × 2, × 4, × 8, × 16, and × 32 respectively, and must be labelled thus.

It is good for the pupils to have experience in making a series of dilutions in this way. To simplify measurement of the dilutions and to avoid the use of measuring cylinders, the test-tubes can be marked beforehand at the 10 cm^3 and 20 cm^3 levels, as suggested above.

Turbidity should occur after six days at room temperature (25°C approximately) but if speedier results are required or if the ambient temperature is less than this, the tubes can be placed in an incubator at about 30°C. Further growth can be prevented by adding one drop of 40 per cent formalin to each tube.

Replication of the set of dilutions will either confirm results or give an idea of experimental error. If results vary widely it will be an indication that some factor has not been taken into account and the experiment should be repeated until they are consistent. Note the colour, smell, and turbidity in each tube before the addition of the indicator.

5. *Indicator*. Brom-cresol-purple is purple in a neutral solution but turns yellow in an acid solution. A 0·004 per cent solution should be used. Only 2 drops of indicator should be added to each of the tubes, including the control. Explain the importance of comparing colour changes in Tubes 1 to 6 with the colour of the control tube, since slight changes in shade are often difficult to detect except by using a comparator. It will be interesting to see if the results obtained with the indicator confirm those of turbidity.

B.D.H. Universal Indicator (obtainable from British Drug Houses) can be used as an alternative. In acid solution it turns red through orange.

6. *Confirmatory test*. Before adding the indicator, cultures of bacteria could be grown on sterilized nutrient agar in sterilized Petri dishes by making streak plates for each of the tubes in the series. Growth of bacterial colonies in some of the plates may confirm the visual results obtained by using turbidity as the criterion.

Optional practicals

In the above experiment other factors can be varied and results compared with those obtained in the class practical.

a. Comparing the action of different disinfectants on soil bacteria. Different phenol-containing disinfectants can be used and results compared. Again a control set will be required (but see the teaching notes to this section).

Lysol clouds very little in solution, turning pale brown at the dilution at which the bacteria start feeding on the phenol content.

The Lysol contains 50 per cent phenol, and a 0·8 per cent solution of Lysol will, therefore, be equivalent to a 0·4 per cent solution of phenol. Thus by adding 100 cm^3 of the inoculum to an equal volume of 0·8 per cent Lysol solution, this will be equivalent to 0·2 per cent phenol.

The principle of replication should be explained, making use of the fact that the more variables there are to be tested at one time, the more replications are required.

b. The effect of different strengths of disinfectant on a pure culture of bacteria.

This experiment is alternative and complementary to the work attempted in section 3.21.

Several Petri dishes are plated out with Blood Agar Base and seeded with a suspension of *B. subtilis* (for procedure, see notes on teaching to section 4.33). Discs of 5 mm in diameter are cut from filter paper and dipped in different strengths of the disinfectant to be tested. Calculate the strength of the strongest dilution by consulting the formula of the disinfectant used. Since most of the coal-tar derivatives contain about 40 per cent phenol, it would be fairly safe to say that the first dilution should be 1/100, and that dilutions of 1/1,000 and 1/10,000 would give a fair range of results. Dip the discs of filter paper in the separate dilutions and place one from each in a marked segment of a Petri dish of agar, seeded with the suspension of *B. subtilis*.

The disinfectant will diffuse outwards from each disc, and if the strengths of disinfectant have been judged right, one disc should be of such a weak strength that no inhibition of the bacterial colonies is evident; the other two on the plate should show zones of inhibition of different sizes, relating to the strength of disinfectant. These zones can be measured in each of the plates and compared.

Factors affecting the efficiency of disinfectants

Some of the factors affecting the rate at which a disinfectant kills micro-organisms are:

a. The strength of disinfectant and the length of time the bacteria are exposed to it. On the whole, low concentrations take longer to be effective and vice versa.

b. The number of micro-organisms present.

c. There may be a number of resistant species present, especially if the total number is large.

d. Many disinfectants are selective and are, therefore, ineffective against many kinds of micro-organisms.

Resistant species

Much has been heard in recent years of resistance by certain pathogens to disinfectants of various kinds. Under certain conditions pathogenic organisms can mutate rapidly to resistant forms whereas the wild type remains non-resistant. This is possibly due to the constant exposure of a species to a certain type of disinfectant, especially in such places as hospitals and laboratories.

The minimum inhibitory concentration

The *minimum inhibitory concentration* (MIC) of a disinfectant (or antibiotic) is the strength of disinfectant at which the bacterial colonies are just inhibited from growth. To make an accurate calculation of the MIC of a disinfectant, it would be necessary to follow a rather different procedure: measured samples of the disinfectant in concentrations ranging from zero to the highest practical concentration are added to a range of tubes containing nutrient broth. The tubes are inoculated with the test organism and an indicator such as methyl red is added. After incubation the tube containing just enough disinfectant to prevent the change from yellow to red of the indicator contains the minimum inhibitory concentration.

Unfortunately we have to accept the fact that the rate of invention of new bactericides is just about keeping up with the rate of increase in the number of resistant species.

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Chapter 4

Man against disease

Objectives

In the last chapter we considered the problem of the control of micro-organisms and the part that antiseptics can play in the process. Now we pass to the prevention aspects of disease, stressing particularly problems relating to the maintenance of human health. This leads on to the cure of disease and the part that chemicals can play in controlling bacterial infection. A particular and outstanding instance is provided by antibiotics and students are given an opportunity of testing the action of penicillin for themselves.

The sequence of development is as follows:

1. The connection between disease and bacteria was established by Pasteur in his work on *Bombyx mori.*

2. Vaccination gives immunity to certain diseases such as smallpox.

3. Some micro-organisms themselves produce substances which destroy others. Such substances are called antibiotics.

4. Sir Alexander Fleming discovered that the mould *Penicillium* inhibited the growth of certain bacteria.

5. By using different strengths of penicillin on a culture of an organism non-resistant to penicillin, it can be shown that the inhibitory zones are of different sizes.

Suggestions for practical work

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4.1
Microbes and disease

Notes on teaching

The fact that the early supporters of the germ theory were concerned with the origin, rather than the significance, of micro-organisms, delayed the development of the theory.

Pasteur's work explained the biological nature of fermentation and it was recognized that this process was similar to the progress of infectious disease. This was supported by the followers of Lister. Although Pasteur, with his work on silkworms, established beyond doubt that micro-organisms can cause disease, the relationship between the two remained far from clear. Koch really put the seal of certainty upon the connection between germs and disease by his successful isolation and culture of anthrax bacilli from spleen tissue of animals suffering from anthrax. Subsequent progress in learning about the bacterial origin of many other diseases was rapid.

Among the bacteria are good examples of all the levels of association between organisms from commensalism to total parasitism. If such an association results in injury or disease to the host, the parasite is said to be pathogenic.

Health and disease are two states which, though complex in their interpretation, are subject to much variation in individuals. Upset of the balance between host and pathogen does not necessarily result in disease.

How micro-organisms cause disease

The question of host resistance to disease is important since the severity of a disease depends upon the reaction of the host as well as upon the violence of the invading organism. The study of resistance has lagged behind that of the causal agents. All this misses one apparent anomaly in epidemic disease, when a percentage of the population appears to be immune to the particular type of infection.

There are two common mechanisms by which disease can be caused by micro-organisms:

a. Mechanical production of disease where the organism interferes with the functioning of tissues in some way, actually blocks vessels. Two examples are the blocking of the water-conducting tissue of plants as a result of bacterial infection and the consequent wilting and death of the plant; and the growth in the tracheal tubes in cases of whooping cough of bacteria in such numbers that they interfere with the proper functioning of the cilia.

b. The production of chemicals. Some bacteria are capable of forming chemical substances or toxins which are poisonous and capable of destroying the defence of the host.

4.2

The prevention of disease

Notes on teaching

Disease and disease-causing organisms make up the negative side of the picture. The history of the discovery of vaccination is an opportunity for the positive side to be emphasized.

The use by Pasteur of weakened, or *attenuated*, cultures of anthrax as inoculants opens up the whole question of immunity, which really comes into discussion following upon the reaction of the body to infection (see section 4.21). But the foundations for this are laid by Pasteur's experiments on anthrax which also provide a further example of the design of experiments. Working on the hypothesis that an attenuated (much weakened) culture of anthrax, used as an inoculant, would produce resistance in the host animal, Pasteur insisted on proof of his hypothesis by a further series of experiments on sheep. Only after such a proof did he permit himself to continue this line of research on inoculation for the prevention of other diseases, culminating in his classic experiments on rabies.

There is no doubt that Koch's isolation of the anthrax bacillus, at about the same time as Pasteur's investigations into the prevention of the disease, greatly assisted Pasteur in the production of his anthrax vaccine.

Edward Jenner (1749–1823)

Jenner was born at Berkeley in Gloucestershire and was himself inoculated against smallpox when he was eight. This primitive method of inoculation was brought to England from the Middle East by Lady Mary Wortley Montagu and involved the injection of the patient with discharge from a mild case of smallpox. The trouble was that in many cases the patient contracted a grave attack of the disease, which usually proved fatal.

Jenner's methods involved the injection of pus, not from smallpox cases, but from someone suffering from the much milder disease, cowpox. It is doubtful if Jenner realized that the causal agent of cowpox is the same, but in a much milder form, as that of smallpox. What he did was to use an attenuated vaccine which produced immunity in the small boy whom he vaccinated. The risk Jenner took in then inoculating the boy with pus from a smallpox case, to prove his point, was considerable.

4.21
How our bodies react to infection

Notes on teaching

An understanding of the phenomenon of immunity requires a knowledge of antigen-antibody reactions, or *serology* as it is called. This is clearly outside the scope of this course and all that is called for is a simple means of interpreting on the broadest lines, the way in which the body reacts to invading organisms.

There are simple questions such as how infecting organisms enter the body, and under what circumstances – over-crowding, damp, and so forth. Are there likely to be large numbers of micro-organisms present? Do invading organisms cause obvious effects in the body such as a rise in temperature?

Application of such knowledge to farming and agriculture can be a focus for class discussion, for modern methods of farming use immunizing agents in the rearing of stock, and of sprays to prevent disease in crops.

Immunity

The defence mechanisms protecting an individual from infection are:

a. The body covering. This includes the skin and mucous membranes which act as barriers to most pathogens, and the hairs in the nostrils with filtered air entering the lungs. The acidity of the gastric juices and such body secretions as mucus trap invading microbes.

b. The body phagocytes. These destroy pathogens by engulfing and digesting them. They include the white blood cells, wandering macrophages, and fixed forms in the lymph, spleen, bone marrow, and liver. Some pathogens escape these protective cells and have to be dealt with in other ways.

c. Immunity. This implies relative or absolute resistance to an infective agent. There are various types of immunity which it is well to understand because this has a direct bearing on the principles of vaccination.

Natural immunity is not the result of previous contact with an infective organism but is an inherited state of resistance. This explains why a few members of a community do not contract an epidemic disease.

Acquired immunity or the development of resistance by an individual is of two kinds.

Active immunity can be acquired naturally as a result of recovery from a disease or artificially by immunization. It is usually a slower but more lasting process, since the individual produces his own immune state by the activity of the body cells.

Passive immunity is the reception by one individual of immune substance produced by another. The immunity conferred is quicker to take effect but may be of comparatively short duration.

Thus we can say that pathogenic organisms can cause disease only when they have succeeded in evading all these methods of defence and manage, under favourable conditions, to establish themselves and to multiply.

4.22
Modern vaccines
Notes on teaching

The fact that the medical profession has accepted vaccination as a necessity in modern practice should emphasize the need for representing vaccination as a routine measure. With modern vaccines the 'pin-cushion' baby is a thing of the past. The Salk vaccine consists of killed virus. Its action is entirely prophylactic, and is similar to that of immunization against typhoid fever, using a suspension of killed typhoid cells.

Diphtheria is probably the disease at which, especially in the first years of life, vaccination is particularly aimed.

The graph in text figure 26 can be interpreted in interesting ways. Omitting years 1940 and 1941, the level is even. During the ensuing ten years, the incidence of diphtheria is down to nil. What happened in the years 1940 and 1941? Could it be that the war reduced the number of available civilian doctors or that the incidence of diphtheria rose, due to war conditions of vaccinating and so forth?

The cause of the periodic decline or rise in the virulence of certain pathogenic organisms is still largely a matter for conjecture, since various factors have to be taken into account. One of the causes is the facility with which both bacteria and viruses mutate to different forms.

4.3
The cure of disease
Notes on teaching

Much has been said about disease-causing micro-organisms but the rest of this chapter will be concerned with those that produce substances which inhibit the growth of pathogens. While penicillin is taken as the best example of an antibiotic, and one which can easily be obtained in the laboratory, it must be remembered that its effectiveness is limited and that in certain cases, resistance to the drug can develop as a result of continuous use.

4.31 The discovery of penicillin: Sir Alexander Fleming (1881–1955)

Fleming was born in Ayrshire into a Scottish family in humble circumstances. He trained under Sir Almroth Wright at St Mary's Hospital and had a brilliant career, becoming Professor of Bacteriology at London University when he was 47. He had already discovered lysozyme, an enzyme capable of destroying the carbohydrate in bacterial cell walls, and it may have been this discovery which helped Fleming, in 1928, to realize the importance of his subsequent observations of the effects of *Penicillium* mould growing on one of his bacterial cultures. The actual extraction of penicillin from *Penicillium* was made thirteen years later by Professor Howard Florey (now Lord Florey) and Dr E. B. Chain in Oxford. The original species of *Penicillium* used was *P. notatum.*

Penicillin

Penicillin is almost completely non-toxic and acts in much weaker concentrations than the sulphonamides. It is also largely unaffected by pus or fluid exuding from tissues. Among the groups of bacteria against which it is effective are *Staphylococci*, *Streptococci*, *Pneumococci*, *Gonococci*, and *C. diphtheriae.* But *Escherichia coli* and *Salmonella* sp. are two common microbes unaffected by penicillin. (See section 4.34.)

4.32 Growing *Penicillium*

Notes on Teaching

Fleming's famous plate probably became infected with *Penicillium* spores from the air. The optional practical shows that soil is a common source of species of *Penicillium* and that it is a fungus, similar in general structure to other micro-fungi.

Optional practical – Growing Penicillium *from soil and from a pure culture*

Apparatus required per class
Pure cultures of *Penicillium chrysogenum*
Per group of four or six
2 Petri dishes
Soil solution
1 test-tube containing 10 cm^3 malt agar in a water bath at 45°C
1 × 2 cm^3 pipette or disposable syringe
1 inoculating loop
2 microscope slides and cover-slips
2 mounted needles
Microscope

The soil solution is prepare by adding about 10 g garden soil to 90 cm^3 distilled water. Shake and transfer 1 cm^3 to 99 cm^3 distilled water to give a dilution of 1/1,000.

The molten agar is poured into two Petri dishes, labelled A and B. Tilt until it covers the base of the dishes completely. 1 cm^3 of the 1/1,000 soil inoculum is added to plate A and rotated carefully to mix the inoculum with the agar (a disposable 2 cm^3 syringe is probably easier to handle than a 2 cm^3 pipette).

Inoculation of the sterile agar in B is made by dipping a sterile inoculating loop into the pure culture of *P. chrysogenum* and placing a small amount of the culture in the centre of the agar. Both plates are incubated at 25°C (room temperature) for three or four days.

The method suggested for growing will undoubtedly result in the production of a variety of micro-fungi including many fast-growing forms such as species of *Mucor*, *Mortierella*, and *Zygorhynchus*, which form dense white mycelia. *Penicillium*, however, grows much more slowly and the colonies may be coloured due to the production of abundant pigmented spores.

The culture of *Penicillium* on plate B, from a pure culture, is recommended since it is possible that no *Penicillium* will be present on A. In any case, B may be a help in the identification of *Penicillium* on plate A.

Pure cultures of *Penicillium* sp. can be obtained in culture bottles from Messrs T. Gerrard & Co., Ltd, Gerrard House, Worthing Road, East Preston, near Littlehampton, Sussex or from suppliers of biological cultures, and if the optional practical in section 4.32 is to be attempted, it may be advisable to stipulate that *Penicillium chrysogenum* of a strain which is active in the production of penicillin is required.

It is not suggested that much time should be spent on the actual identification of *Penicillium* on plate A. It would be better either to make a temporary slide of *Penicillium* from the pure culture or to use prepared slides, as demonstrations of micro-structures to be used with text figure 29. The question may, however, arise as to why in A no sterilization was needed for either dish, agar, or inoculating loop, whereas this was necessary for the inoculation of the pure culture.

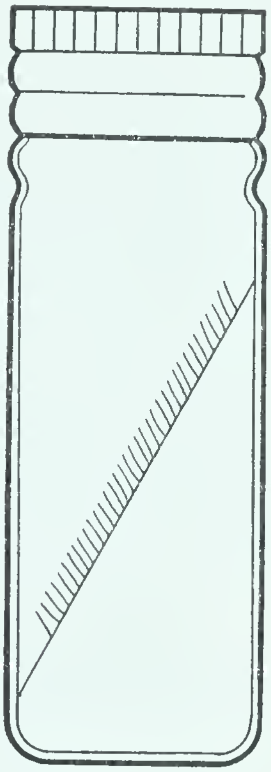

Figure G2.
'Universal' culture bottle containing agar slope on which a stock fungus culture is growing.

Preparation of malt agar

Malt Agar is cheap, easily made up and useful for all elementary work. Malt extract, obtainable from most chemists, contains all the nutrients required for growth of fungi. Weigh 2 g malt extract in a small beaker, dissolve in hot distilled water and make up to 100 cm³ to give a 2 per cent solution. Put this in a 250 cm³ flask and add 2–2·5 g of powdered agar. Cover mouth of flask with a loose plug of cottonwool which is covered with tinfoil, and then autoclave the flask and contents.

Malt Agar, if not prepared in the laboratory, can be obtained in bottles ready made up from Messrs Oxo Ltd (see above). The same firm supplies Sabouraud Maltose Agar, which has a pH of 5·2.

Maintaining stock cultures of micro-fungi

Stock cultures are usually kept on slopes of medium in plugged tubes or, better still, in 'Universal' culture bottles (see figure G2). These are made by placing the bottles containing the molten sterilized medium at an angle until they are cold. The advantages of using a 'Universal' bottle become apparent during the sterilizing operation. Not only do they not need plugging, having a screw top and liner, but they will also stand on their own inside the autoclave.

After inoculation, the slope cultures should be examined after two or three days to make sure that the fungus is growing satisfactorily and is not contaminated. The bottles can then be labelled and stored. Stock cultures are usually transferred every three or four months.

Making slide mounts

a. A slide mount is necessary for investigating the structure of moulds, but temporary slides are quite easy to make for this purpose.

Lacto-phenol is the most useful general mountant and is made by dissolving 20 g of phenol crystals in 20 g warm distilled water and adding 20 g lactic acid and 40 g pure glycerol, to which cotton blue (50–100 mg in 100 cm^3 lacto-phenol) can be added as a stain.

A drop of lacto-phenol is put on a slide and a small portion of the fungus culture put into the drop. Cover with a clean cover-slip. Such a preparation will not dry out for several weeks.

b. Even with the greatest care in removing a portion of culture for mounting, the mycelium and fruiting bodies may be damaged. For such delicate structures the following method is recommended.

Soak a filter paper in 30 per cent glycerol and place in the bottom of a sterile Petri dish. A clean sterile slide is supported on two short lengths of glass rod above the filter paper (see figure G3). Cut a small block of malt agar from a poured plate and place on the microscope slide. Inoculate the sides of the block with the pure culture and place a cover-slip on the top. The fungus will grow and extend into the moisture on the cover-slip and slide. After several days the cover-slip may be lifted off and the agar block removed. Two mounts can then be made, one with the slide and one with the cover-slip, using lacto-phenol and cotton blue as the mountant. This method involves the minimum disturbance of the mycelium.

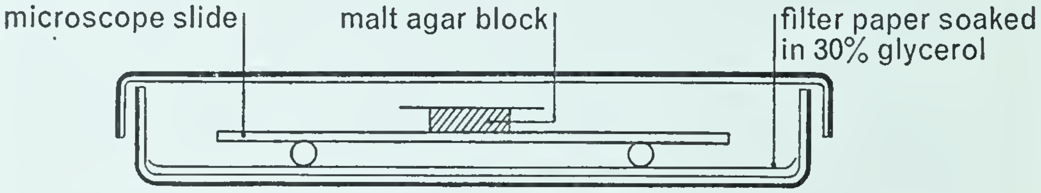

Figure G3.
Growing a fungus culture on a microscope slide.

Extraction of penicillin

Penicillin is produced commercially by submerging cultures of *P. chrysogenum* in large fermentation vessels which are provided with a vigorous aeration. One of the chief difficulties of production is the prevention of contaminating organisms, since many bacteria can produce the enzyme penicillinase, capable of destroying all the penicillin in a culture vessel.

Large numbers of spores are obtained by growing the mould on solid media. They are then germinated in small fermenters, where they form spherical mycelia. The mycelial culture is then transferred to the main fermenters containing a medium composed of corn-steep liquor (an aqueous corn extract), sugar and mineral salts. Under submerged culture conditions a benzyl compound of penicillin is formed. The medium is supplemented with substances such as phenyl-acetic acid which increase the yield of penicillin. The culture is maintained for three days at 26°C, when the mycelium is filtered off and organic solvents are added to extract the penicillin. Further purification involves converting penicillin into a sodium salt which is crystallized out.

4.33
Penicillin as an antibiotic

Apparatus required per class
Discs impregnated with *Bacillus subtilis*
Nutrient Broth No. 2
Per pair of students
1 sterile Petri dish plated with sterile Blood Agar Base and seeded with a suspension of *B. subtilis*
Chinagraph pencil
1 pair blunt forceps
1 × 5 unit penicillin disc
1 × 1·5 unit penicillin disc
Sellotape

Notes on teaching

Penicillin has been chosen to demonstrate its antibiotic effect on certain organisms. By using an inoculum of *Bacillus subtilis*, a non-pathogen, and a paper disc impregnated with penicillin, clear zones of inhibition are obtained after incubating for 18 hours at 37°C.

1. The sterile Petri dishes are plated out with sterile Blood Agar Base in the usual way and then allowed to set firmly before they are seeded with a suspension of *B. subtilis*. This is made by adding one disc impregnated with *B. subtilis* to 10 cm^3 sterile Nutrient Broth No. 2 in a McCartney bottle. This amount will be sufficient to flood two plates. To prevent the entry of contaminants, the disc should be handled with sterile forceps and the screw cap replaced on the bottle immediately. The culture is then incubated at 37°C for 24 hours and agitated several times during this period. Clumps may form in the culture so that a final shaking is needed after which it should be allowed to settle for 15 minutes.

2. About 5 cm^3 of the supernatant fluid is withdrawn from the bottle with a sterile pipette and this is used for flooding a plate prepared in (*1*) above.

3. Tilt so that the whole surface becomes wetted.

4. Remove the excess suspension by aspirating off with a pipette.

5. The excess fluid can then be used to seed another plate, adding further suspension as required.

6. Unwanted suspension is finally discarded into a Lysol pot.

7. Dry the plates with the lids off and in an inverted position in an incubator at 37°C until no moisture is visible. This should take about an hour.

After the class has placed the penicillin discs on the cultures (keeping the writing '1·5 i.u.' and '5 i.u.' uppermost), these should be incubated for eighteen hours at 37°C. A short period of incubation at this temperature is necessary for sensitivity testing to avoid the prolonged diffusion period that would arise if the organisms were to grow slowly at room temperature. To preserve the plates after the period of incubation and until the next class period, a circle of filter paper, onto which are pipetted two or three drops of formalin, can be placed inside the lid of each Petri dish. The dishes should then be kept in an *inverted* position.

Materials required

The Blood Agar Base, the *B. subtilis* discs, and the penicillin discs can all be obtained from Oxoid Ltd (see above).

The penicillin discs should be kept in a refrigerator until they are required. For class purposes it may be best to extract, from the stock bottles, the number needed of each strength and hand them out in plugged sterile tubes.

Although these cultures are no more potentially dangerous than many other hazards encountered in a laboratory, it is nevertheless advisable, since the bacteria listed below are pathogens, to insist upon certain precautions:

a. The pupils should inspect and measure the colonies from the *back* of the plate, all lids being secured by Sellotape to the base.

b. After use, all used cultures should be immediately placed in a container of 10 per cent Lysol (or 5 per cent Sudol).

The instructions given in the text for making and labelling the plates should be followed carefully, since it is important to know the strength of each disc. Positions on the plate can easily be transposed while they are being placed on the agar.

4.34
Other kinds of antibiotics

Testing the sensitivity and resistance of organisms to penicillin and streptomycin

Materials required for the demonstration

Nutrient Broth No. 2
Blood Agar Base
1 *Staphylococcus albus* disc
1 *Escherichia coli* disc
2 penicillin discs (5 i.u.)
2 streptomycin discs (25 μg)
1 inoculating loop
1 pair forceps

Notes on teaching

The class experiment in section 4.33 has demonstrated the ability of the antibiotic penicillin to inhibit the growth of the organism *Bacillus subtilis*. But penicillin may not be an effective antibiotic against all types of bacteria. On the other hand, an organism may be inhibited by more than one kind of antibiotic. This can be tested by using the two antibiotics, penicillin and streptomycin. There are very few bacterial diseases which do not respond to one or other of these drugs.

The use of paper discs impregnated with the antibiotics has made the experiments easy to conduct. Similarly it is possible to obtain paper discs impregnated with different bacteria and from which cultures can easily be made. The impregnated discs should be stored in a refrigerator.

Procedure

1. Make up two tubes of sterile nutrient broth, 10 cm^3 each, marking one '*S. albus*' and the other '*E. coli*'.

2. Inoculate the '*S. albus*' tube with a disc of *S. albus*, and the '*E. coli*' tube with a disc of *E. coli*. Incubate at room temperature for forty-eight hours before the experiment.

3. Pour and set two plates of Blood Agar Base, labelling one '*S. albus*' and the other '*E. coli*' with a Chinagraph pencil on the base of the dish.

4. Plate out two loopfuls of the *S. albus* broth culture (made in *2* above) on the plate marked '*S. albus*' and two loopfuls of the *E. coli* broth culture on the plate marked '*E. coli*'.

5. On each plate place one penicillin disc with the writing '5 i.u.' uppermost and one streptomycin disc, one to two inches apart and not near the side of the dish. With a Chinagraph pencil, label the position of each on the base of the dish. Incubate at 37°C for eighteen hours and store in a refrigerator until required. (The temperature of incubation is important for good results.)

Results expected

If either organism is sensitive to an antibiotic, a zone of inhibition will appear round the disc.

	Penicillin	Streptomycin
E. coli	Resistant (no inhibition)	Sensitive (inhibition)
S. albus	Sensitive (inhibition)	Resistant (no inhibition)

All the materials required for this experiment are obtainable from Oxoid Ltd (see above).

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Further reading

*=reading for pupils

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Chapter 5

Shapes, sizes, and movement

Objectives

The purpose of this chapter is to introduce the idea of quantitative relationships in the structure of living organisms, and the part they play in influencing the kind of life that different animals and plants can lead.

The development of the chapter is as follows:

1. One of the principal problems facing animals is that of support.
2. The larger an animal becomes the greater are the problems of support that it must overcome.
3. For vertebrate animals, the dimensions of their bones are of particular importance. What determines the strength of a bone? How is its strength affected by its length and cross-sectional area?
4. Small animals also face peculiar problems which derive mainly from their relatively large surface area.
5. Animals living in water have to overcome its resistance to movement. This has resulted in the adaptation of various forms of streamlining.
6. Plants also have to overcome problems arising from their size, shape, and mode of life. The chapter ends by contrasting the mode of support of plants and animals, and the ways in which their respective requirements have been met.

Suggestions for practical work

			Page	
	Section		*Text*	*Guide*
Class practical work	5.11	Size and support. Relationship between volume (weight) and linear dimensions Relationship between cross-sectional area and support	56	50
	5.12	Strength and length. Length and ability to support weight	61	54
	5.13	Bones. Length and cross-sectional area related to weight supported	64	55
	5.14	Weight in water	65	56
	5.21	Construction of a streamlined model	69	58
	5.22	Stability by splaying out legs	72	58
Demonstrations	5.11	Models illustrating linear dimensions (for those who cannot follow the mathematical arguments)	56	50

	The use of cartridge paper models to show load bearing capacity		
5.12	The use of large paper tubes to show relationship between length and strength	61	54
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5.21	The speed of travel of various shapes in water	69	58
5.22	Achieving stability by splay supports	71	58

Notes on teaching

Students will already be aware of a bewildering diversity of size and shape in living things. They will also have a general idea of the proportions of the parts of large plants and animals. The reality of seeing the huge bulk of the larger animals is sufficiently important at this stage to justify a visit to a zoo. In general terms students should look at thickness of limbs, stocky build, short limb sections, laboured movement, and inertia. Most large cities have specimens of whales in their museums. The Whale Gallery and Large Mammal Section at the Natural History Museum, South Kensington, are well worth a visit. If this cannot be arranged in term time, one or other of the above visits should be suggested for the holidays preceding this work.

Books of the 'Wonders of Nature' or 'Records' type in the children's section of a library, for example the *Guinness Book of Records*, can provide useful information.

Discussion on what is gathered from all these sources can provide a valuable lead-in to the precise measurements which are used in this chapter to establish numerical relationships.

Opportunity should be taken throughout the following chapters to distinguish between the several different ways in which measurements may be reported. In particular, students should be aware of the following categories of measurements:

1. To quote a single measurement of size is permissible only when this refers to the size of a single individual or to a class of individuals all with the same size. Thus, 'This man is 6 ft 0·6 in tall' and 'Halfpennies are 1·0 inch in diameter'.

2. To describe the size of a class of individuals which differ from each other, it is customary to quote the average of a representative series of measurements. Thus, the statement 'The average height of Englishmen is 5 ft 7·5 in' can be based on a sufficient number of measurements of the height of individual Englishmen.

3. For a class of individuals differing among each other, it is also necessary to have some means of suggesting how great may be the extent of the variation between individuals. There are a great many ways in which this can be done, and at this stage students need merely an appreciation that it is usually important to indicate the range of variation when an average value is quoted. Thus the statement 'The average height of step-ladders in England, like the average height of Englishmen, is 5 ft 7·5 in', even if it were true, would, on its own, conceal the fact that the variation of the height of step-ladders is probably greater than that of Englishmen. At this stage it is sufficient that students should be aware of the importance of – and familiar with – statements such as: 'Two-thirds of all Englishmen are between 5 ft 5 in and 5 ft 10 in tall.'

Students should also understand the limitations of their apparatus. Measurements with a ruler are accurate to only 0·5 mm.

The experiments and demonstrations have been devised so as to arrive at the numerical relationships accurate to the nearest whole number. Details of mechanics and precise definitions of physical terms have been avoided. What is important is to derive those relationships which are of biological significance.

The problems posed make it inevitable that models should be used, but every opportunity should be taken to relate the conclusions from experiments with models to the situation in living things.

5.11
Size and support
Demonstration experiment

Apparatus
Rulers with cm and mm markings
Razor blades or scalpels
Plasticine
Spring balances 0–100 g, 0–250 g, 0–500 g, and 0–5 kg
Boards and dowels for rolling out Plasticine cylinders

For classes of students who already have a clear idea of the relationship between linear dimensions and volume this class experiment may be unnecessary. Where mathematics teaching is closely related to science the relationship will already have been established, and a quick demonstration will be sufficient to remind pupils.

Class experiment

In practice it is difficult for children to cut 1 cm and 2 cm cubes accurately but a good result can be obtained by allowing some of the class to make cubes and then weighing one whole batch together to get the average weight for each size. It is essential to provide spring balances with ranges 0–100 g, 0–250 g, 0–500 g and 0–5 kg for this experiment, and for the next one in which cylinders of Plasticine are crushed.

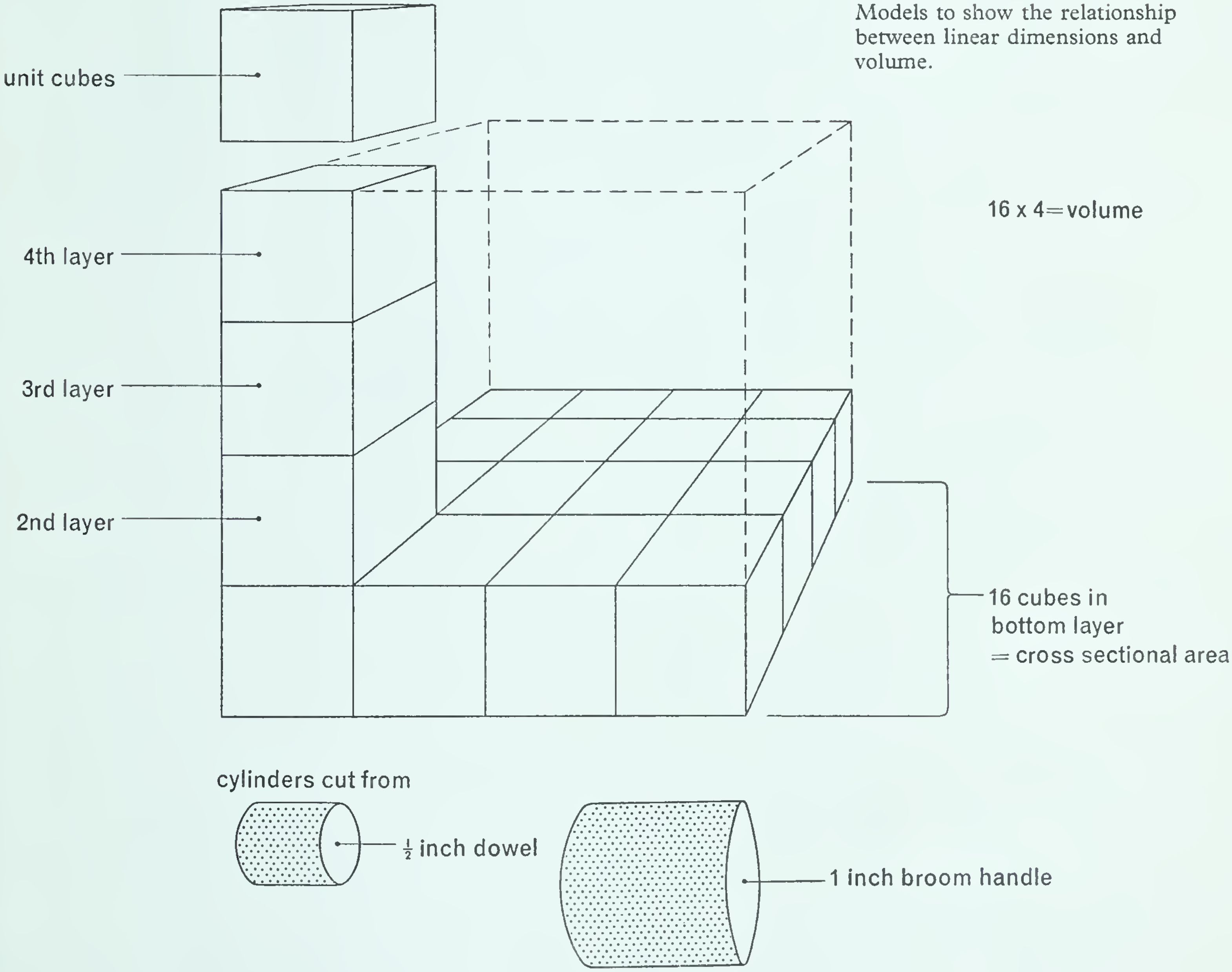
The class experiment, using cubes of Plasticine, could be replaced by a quick demonstration using wooden blocks, sawn from prepared timber, and sanded down to size. Taking a 1 inch cube as the unit, other cubes of linear dimensions 2 inches, 3 inches, and 4 inches, show clearly how the area of one face and the volume of the cube increase with linear dimensions. For non-mathematically minded pupils it may be necessary to have about 20 unit cubes to build up the base layer and the height of each of the larger cubes (see figure G4).

To demonstrate that the same ratios obtain for cylinders, you could cut models of these from $\frac{1}{2}$ inch dowel (in $\frac{1}{2}$ inch lengths) and from a sanded down broom handle (sanded to 1 inch diameter and cut in 1 inch lengths).

Special mathematical models and charts to demonstrate these relationships can be bought. Transparent plastic models are ideal but expensive.

Plasticine is the most convenient medium for these experiments as it can be used repeatedly and if worked until soft does well for weighing and cutting.

Figure G4.
Models to show the relationship between linear dimensions and volume.



Uniform rods of Plasticine are best prepared by rolling them between two smooth boards. The diameter is gradually reduced by gently rolling the top board over the Plasticine. Two dowels ($\frac{1}{4}$ inch, $\frac{1}{2}$ inch, and $\frac{3}{4}$ inch if millimetre sizes are not available) between the boards act as spacers to give the final diameters (see text figure 34). The uniform part of the rod is then cut into measured lengths for each pupil. A ruler can be used by pupils as an upper rolling board to restore each cylinder to its original length for quick repetitions. The 'feel' of the Plasticine as it begins to collapse when pressed downwards on the pan of the household scale type of balance is a reasonable indication of the stress point. As the rod thickens the pressure needed to make it collapse further increases rapidly. It is important to take the reading at the beginning of the collapse.

More uniform results are obtained using as a height gauge a tubular collar of slightly greater diameter than the Plasticine cylinder. See text figure 33.

With soft Plasticine in a warm room the 5 mm rod begins to crush at 100–150 g (4–6 oz). You get the same result on the 1·0 mm rod when the pressure is about 400 g.

Absolute diameter is not vital, provided the rods of Plasticine supplied to students have diameters which are two, three, and four times the diameter of the thinnest rods (controlled by the dowel rod).

To repeat the experiment, students re-roll the Plasticine to the length originally supplied.

Demonstration experiment

An alternative to the Plasticine experiment to show supporting function of a given cross-sectional area:

A tube of cartridge paper is used in the experiment. The cross-sectional area cannot be measured, but it is reasonable to assume

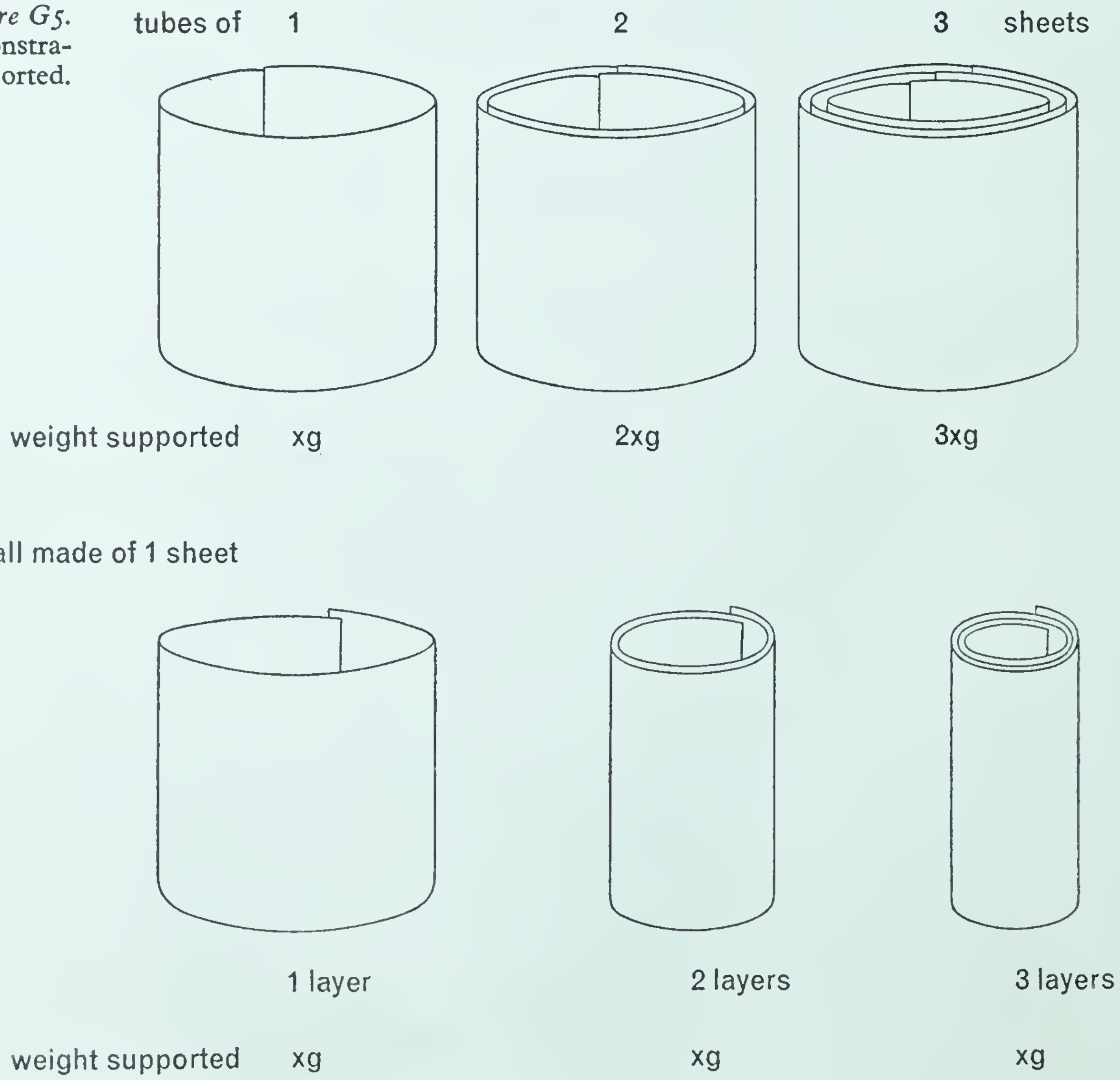


Figure G5.
Tubes of paper for the demonstration of weight supported.

that equal lengths of paper forming the tube will give equal areas. The tubes must be prepared at least a day before by gumming the edges of each length of paper. This introduces an extraneous influence – the thin layer of gum – but the results are not noticeably affected.

A standard half-imperial sheet of drawing paper forms a useful unit as the tube made from it fits inside the rim of a galvanized iron bucket. It is better to use poor quality paper, so that the load needed to collapse the tube should not be too great.

Prepare the following tubes:

1. Single sheet of paper
2. Two sheets of paper
3. Three sheets of paper

These will clearly have cross-sectional areas in the ratio of 1:2:3 units of the paper making up the wall of the cylinder.

The bucket is placed squarely on the top of the paper roll and a pupil grasping the handle holds it lightly in position. No weight should be taken on the handle, the support being there to catch the bucket when the paper cylinder collapses. When this happens, the bucket plus sand is weighed (figure G5).

Is the weight supported proportional to the amount of the paper in the wall of the cylinder? Pupils may argue that it is due to three thicknesses being stronger than one.

Three tubes should be prepared using one sheet of cartridge paper for each tube:

1. A single thickness.
2. A sheet cut in two lengths to make a double-walled tube.
3. A sheet cut in three lengths to make a three-walled tube.

Suitable tins should be found to fit the tops of each of these tubes. Wire handles fitted to the tins assist in support and weighing. Each tin is filled with enough sand to collapse the tube on which it is placed.

In this demonstration it is important to load the tins evenly. While it is true that a given cross-section of paper will support a fixed load no matter how the paper in the tube is rolled, the same is not true of the bending strain on the tubes of different diameters.

Mathematical treatment

If a mathematical treatment is required it can be developed for the cube and cylinder:

Cube. Length $=l$; cross-section $=l^2$; volume $=l^3$.

The volume can be taken as a measure of the weight, for the weight is proportional to the volume. Doubling the dimensions to $2l$ gives a cross-section of:

$(2l)^2=4l^2$ and a volume of $(2l)^3=8l^3$. Take l as 1 inch or 1 cm.

Cylinder. This shape is important because most animals are cylindrical rather than rectangular, and it is well to show that the same ratios apply when dimensions are doubled.

Length $=l$ Diameter $=\mathrm{d}=2\mathrm{r}$ (when $\mathrm{r}=$ the radius)

Cross-sectional area will be $\pi\mathrm{r}^2$

Volume = area of end × length $=\pi\mathrm{r}^2 l$

Doubling the dimensions to $2l$ and 2d gives a cross section $\pi (2r)^2 = 4\pi r^2$ and a volume of $\pi(2r)^2\, 2l = 8\pi r^2 l$.

At this stage students should have discovered that weight-bearing capacity depends on the cross-sectional area. When the diameter of a column of Plasticine is doubled, it will support four times the weight before it squashes, because there is four times as much Plasticine in any one place to be squashed.

With the paper rolled into a tube, two lengths will support twice the weight because there is twice as much paper to be compressed. The equivalent of doubling the diameter of the Plasticine would be to use four sheets of paper, that is twice the width and twice the thickness.

The point that must be understood clearly is that *the weight of an animal increases as the cube of its dimensions, whereas the support only increases as the square of dimensions.* Therefore to maintain the material needed for support, legs must increase in diameter more quickly than the animal increases in length.

5.12
Strength and length

Apparatus

Milk straws
Retort stands and clamps
Spring balances as for section 5.11 or
Light scale pans and weights

Waxed milk straws are a most convenient standard material for this experiment. They crack and break quickly when the loading reaches a point at which they start to bend. Select only the unbruised straws.

The milk straws are most conveniently supported in clamps at each end by a piece of dowel or a cork borer fitting tightly inside. Alternatively a nail projecting from a piece of wood held in a retort stand does equally well. There should be either 16 cm, 8 cm, or 4 cm of straw between the two supports. The load can be applied to the centre of the straw either by using a very light scale pan and gram weights, or by using a spring balance attached by a wire loop. A 16 cm length of straw will break when the load is in the region of 100–150 g. The 4 cm length usually requires a load of 500–600 g. As there is considerable variation between individual straws, it is necessary to take the average of a number of results.

The experiment could well be repeated with living plant material – narcissus stems, dandelion flower stalks, or willow twigs. It is obviously necessary to select specimens of uniform diameter.

Dead plant material is also suitable, hogweed stem, for example.

Demonstration experiment

A demonstration using paper tubes can be substituted for the milk straw experiment. The tubes are conveniently made using a broom handle as a former. Wind cartridge paper round the handle to give a single thickness of paper plus a quarter of an inch overlap. Poor quality paper should be used so that the tubes will not be so strong that a heavy load is needed to make them collapse. A number of strips of the same length are prepared and lightly gummed on the overlap to form tubes. They must be prepared at least one day before use to allow the joint to dry. Prepare tubes 20 inch, 10 inch, 5 inch and $2\frac{1}{2}$ inch long of a single thickness of paper and also 20 inch tubes of 2, 3, and 4 thicknesses of paper. To hold the tubes, cut two pieces of broom handle about $2\frac{1}{2}$ inch long and push one into either end of the tube for not more than $\frac{1}{2}$ inch. The broom handle can then be firmly clamped in a retort stand. The tubes are weighted at their centre by a loop of $\frac{1}{2}$ inch tape attached to the handle of a can or bucket. Sand is poured into the bucket until the tube collapses. The bucket plus sand is weighed (see figure G6).

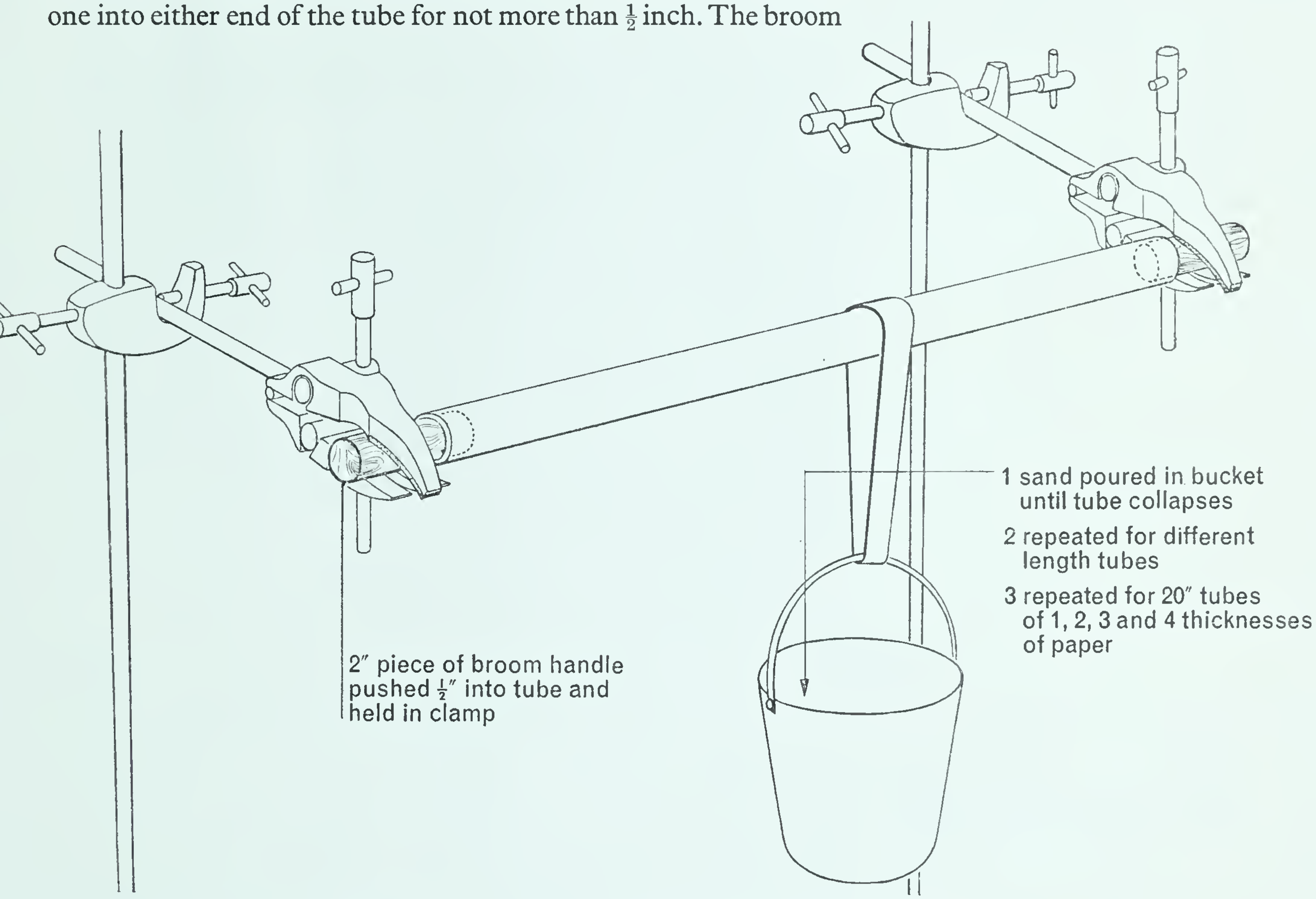

1 sand poured in bucket until tube collapses
2 repeated for different length tubes
3 repeated for 20″ tubes of 1, 2, 3 and 4 thicknesses of paper
2″ piece of broom handle pushed ½″ into tube and held in clamp

Figure G6.
Demonstration of the weight required to bend paper tubes of varying lengths.

Materials required

Prepared leg bones (femur) of bullock, sheep, rabbit, chicken, and man, for measurement of length and diameter

5.13
Bones

For most students there will be no time to extend this section beyond what the text suggests. The students should be able to apply what they have discovered in the previous experiments to their examination of the leg bones of widely different animals.

There are, however, a number of other examples which could be introduced at this stage. The relationship between the size and thickness of the material used as support is well illustrated in animals with exoskeletons. A collection of mollusc shells will show that the larger shells are much thicker in proportion to size than the smaller. Compare mussel and oyster, winkle and whelk. Arthropods also provide good examples if you compare the thickness of chitin of small and large insects or the shell of a crayfish and a large crab. Homely experience of paper bags is useful in comparing the tissue paper sweet bag that holds 4 oz with the sugar bag that holds 2 lb and the fertilizer bag for 1 cwt. These are not just thicker in proportion to their linear dimensions. A further extension, if time permits, can be carried out using the following demonstrations suggested by L. C. Comber Esq., H.M.I.:

To pose the problems, get two large femurs of a bullock from the butcher. Saw one longitudinally and the other into two rings by transverse cuts. The deposition of bone can be seen and measured. Why is the bone not uniformly thick?

If you make a bundle of 20–30 milk straws, held together by an elastic band at each end, and stand it up and then strike it sharply on top, all the straws will fracture at the centre. Bones do not fracture in this way and the reason for this can be seen by examining the sections of femur. These show clearly that there is more bone in the mid-length position. This strengthening effect can be demonstrated using a tube of paper similar to that in section 5.12. It will be found that the tube of two thicknesses bends less easily than the tube of one thickness.

Now prepare tubes with a double thickness only in the centre half and a treble thickness in the centre quarter. Compare the load which these will take with the 1, 2, and 3 thickness complete tubes. Clearly, an economy of material is achieved by strengthening the part which takes the most strain and would collapse first. The distribution of bone follows this principle (see figure G7).

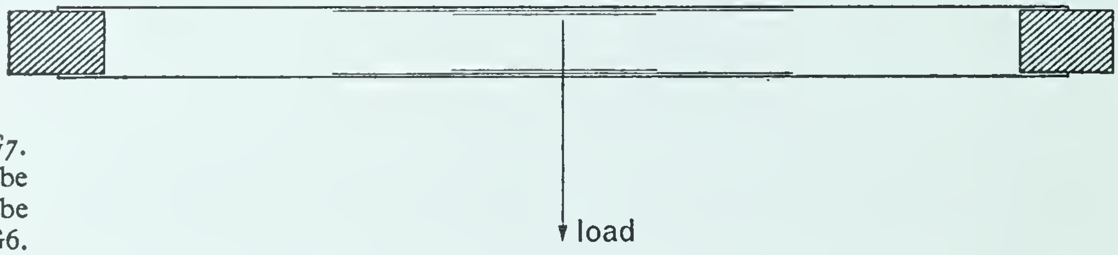


Figure G7.
Load bearing by a paper tube reinforced at the centre. This tube is set up as in figure G6.

5.14
Weight in water

Suitable aquatic plant material for this experiment includes the underwater parts of the broad-leaved pondweed (*Potamogeton natans*), water crowfoot (*Ranunculus fluitans*), starwort (*Callitriche* sp.) and Canadian pondweed (*Elodea canadensis*).

These may be compared with typical land plants such as privet or groundsel.

Even more convincing for such comparisons are beds of seaweed, either *Fucus* or *Laminaria*, which are supported immersed but which collapse completely when left stranded on the shore.

5.15
Smallness

At this stage it is sufficient to draw attention to smallness without attempting to relate it to the factors which determine the minimum size for any living thing. These factors differ for plants and animals with different organizations. The examples that pupils are asked to investigate should convey the idea that the minimum size for some groups is in fact comparatively great. Warm-blooded animals have a relatively large minimum size and the reason for this will become apparent in later work. In fact, the smallest warm-blooded animal is large compared with the largest animal of some invertebrate groups. Some pupils will have seen books and programmes involving microbiology, and may ask questions about the smallest of living things in terms of molecular biology. It may be possible to introduce the idea of the smallest group of molecules which can function independently, particularly if the students have seen Brownian movement. The teacher need not introduce either microscopic organisms or molecular biology unless the pupils ask questions which require explanation by reference to molecules.

All molecules are smaller than the wavelength of light, so that anything that can be seen with an optical microscope must consist of many molecules. Atoms and molecules are in rapid movement, and we can see the effect of this on the smallest particles visible to us. Fine particles of smoke appear to 'dance' in a beam of light. Other small particles in water can be seen to be 'dancing'. This is Brownian movement. For any organism to operate predictably, it must be big enough for the disorderly jumping caused by the movement of nearby atoms or molecules to be unimportant.

The molecules of living things are large and complex, yet they cannot be seen directly by an optical or electron microscope. An electron microscope, which has a range of magnification starting at several thousand diameters and reaching 100,000 diameters can show the smaller bacteria and most of the viruses. Some of the virus particles consist of small numbers of complex single compounds. Others – for example, influenza viruses – are much more complicated. Living things which are big enough for a variety of structures to exist within them come at the lower end of what can be seen with the optical microscope.

5.16
The chessboard of life

The investigation using the chessboards can lead to a good deal of research into books of records, encyclopaedias, and biology books of all kinds. Weights can range through tons, pounds, ounces, grams, milligrams, and finally microscopic measurements. Useful check points are square 11 – the largest wild British mammal (Scottish red deer, stag).
Square 13 – man.
Square 26 – smallest mammal (In Britain – the pygmy shrew).
(Man is about halfway between a mouse and a giant whale.)
Square 38 corresponds to an animal of the same weight as the smallest in an ordinary weight box – 1 mg.

The smallest animal which would turn a delicate balance would be on square 45 and would weigh 0·000001 g. Below this we have to use measurements of dimensions. The seeds of orchids are in this range; the spores of fungi are well below it. Even when we reach square 64 we have not yet reached the size of the smallest living thing.

5.21
The streamlined form

Apparatus required

Long tubes (5 ft) 1 inch diameter with rubber bung
Plasticine
Lead shot or ball bearings 0·5 g

The drag of water on an object such as a bucket is really dramatic. It should be easy to establish that a torpedo-shaped object, for example, a pupil in the swimming pool with arms and legs outstretched, moves very much more effectively in water than the bucket on the end of a rope. Gliding positions assumed during swimming can be related to the streamlined form. It is also easy to show that projections impede progress, for instance by the pupil spreading out his arms and legs. The harness and rope used in a teaching bath are useful for this experiment. Try posing the question, 'How can an earth satellite travel at the speed required to orbit the earth in ninety minutes even with antennae and solar radiation collectors projecting?'

For pupils interested in motor racing or aircraft the development of the streamlined form is readily shown in the history of design. The older aircraft had wing struts almost circular in cross-section. These were superseded by oval and finally by struts streamlined in section. Modern enclosed aircraft are streamlined throughout. Racing cars, too, have undergone similar changes as speeds have increased. The most obvious is the development of the long tapering 'tail' and a similar taper behind projections – cockpit, cowls, engine housing, and spare petrol tanks.

Class assisted demonstration

To time models sinking in water it is necessary that they should move fairly quickly. If less dense materials are used, they may sink so slowly that the effect of their shape is not measurable. The easiest way to compare speed is to have two similar tubes 1 inch in diameter and about 6 ft long: both are corked and filled with water. Pairs of models can then be released simultaneously and the class can see which sinks the faster. Polycell may be used in the water to slow down the movement.

With the 1 inch diameter tube you can use models each made from 1·0 g of Plasticine weighted with 0·5 g lead shot. For class use the long, wide tubes can be replaced by pairs of measuring cylinders. Accurate timing is then impossible, but students will notice differences in the time of hitting the bottom. You must hold the models just touching the surface of the water and release them simultaneously.

5.22
Shape and support

Apparatus required

Plasticine and milk straws (4 per group)

Pupils should be asked to relate the models to such long legged animals as they may have seen. A newly born foal, an adult gazelle, and a giraffe all show the splayed leg stance which is so essential to stability. Invertebrates such as the daddy-long-legs and harvestman, have long legs of slender construction. These are only serviceable because they carry a light load. Their wide spread provides a stable base. Further development of such ideas can be associated with the upright posture of men. The use of arches braced by muscles, tendons, and ligaments plays an important part in maintaining position. Man is not very stable, and has to rely on postural reflexes to remain balanced.

Further work on shape – long term project for groups of pupils

The shape of animals which have to maintain their position in swiftly flowing streams or on wave-swept rocks is a revealing study. The animals are mostly 'limpet shaped' with the flat side closely held to the substratum either by suction or appendages, and have a humped back to divert the flow of water. Any appendages do not project. Contrast this with plants which are feathery and which 'give' with the current.

Bibliography

References

As size, shape, and movement are so intimately connected the references are summarized to cover the whole term's work. Most of them have, in fact, some reference to each of the Chapters 5, 6, and 7.

Further reading

*=reading for pupils

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Chapter 6

Size and surface in living things

Objectives

In Chapter 5 pupils will have gained experience of measuring various living things and will be familiar with the ideas of linear dimensions, surface area, cross-section, and volume. In this chapter we are concerned only with surface area, and the sequence of the material is as follows:

1. The surface of animals and plants varies both in its extent and structure.

2. The question is then asked whether there is a relationship between the surface of an organism and its volume. To what extent is this relationship altered if the surface of a body has large projections, e.g. the large ears of an elephant?

3. To what extent are heat loss and uptake related to surface area? – An important consideration for both warm- and cold-blooded animals.

4. Is surface area related to water uptake and water loss? How do these factors affect the lives of different kinds of animals living in different places?

5. From such considerations can we infer what sorts of shapes and sizes best suit a particular plant or animal living under particular conditions?

Suggestions for practical work

	Section		Page *Text*	*Guide*
Class practical work	6.11	Making cubes of different volumes Comparison of surface area of a mouse and a plant	83	61
	6.12	(Optional) Surface/bulk relationship of a human being	83	63
	6.21	Heat loss from objects of different volumes	85	65
	6.3	Water loss through different surfaces	89	66
	6.31	Investigation of waxes and oils to prevent evaporation	91	67
	6.32	The weight of water held by a surface Water-repellent substances	92	67
Demonstrations	6.12	Comparison of the surface area of a smooth and fluted cylinder. Increase of surface area due to irregular shape in a bunch of grapes	83	63

6.22	Effect of insulation on cooling	86	66
6.23	Relationship between surface and weight of water held using felt covered cubes	88	66

6.11
Weight, volume, and surface area
Notes on teaching

Apparatus
Cardboard to make cubes of various sizes or a selection of rectangular boxes of widely differing sizes
Rulers marked in inches and centimetres
Models of objects of similar volume but different shape

It is worth encouraging pupils to make cubes of various sizes from cardboard to emphasize the relationship existing between surface area and linear dimensions. Some pupils may be able to calculate the sizes of the three cubes of which the linear dimensions, surface area, and volume, respectively, are double those of a standard cube. (These also provide striking proof of the need to state precisely what we mean when we say one thing is twice as big as another.) For those not able to calculate size, give the length of side for the × 2 cubes.

1 in^3	side	1 in
× 2 linear	,,	2 in
× 2 surface area	,,	1·414 in
× 2 volume	,,	1·26 in

The × 2 volume cube looks very little larger than the unit cube.

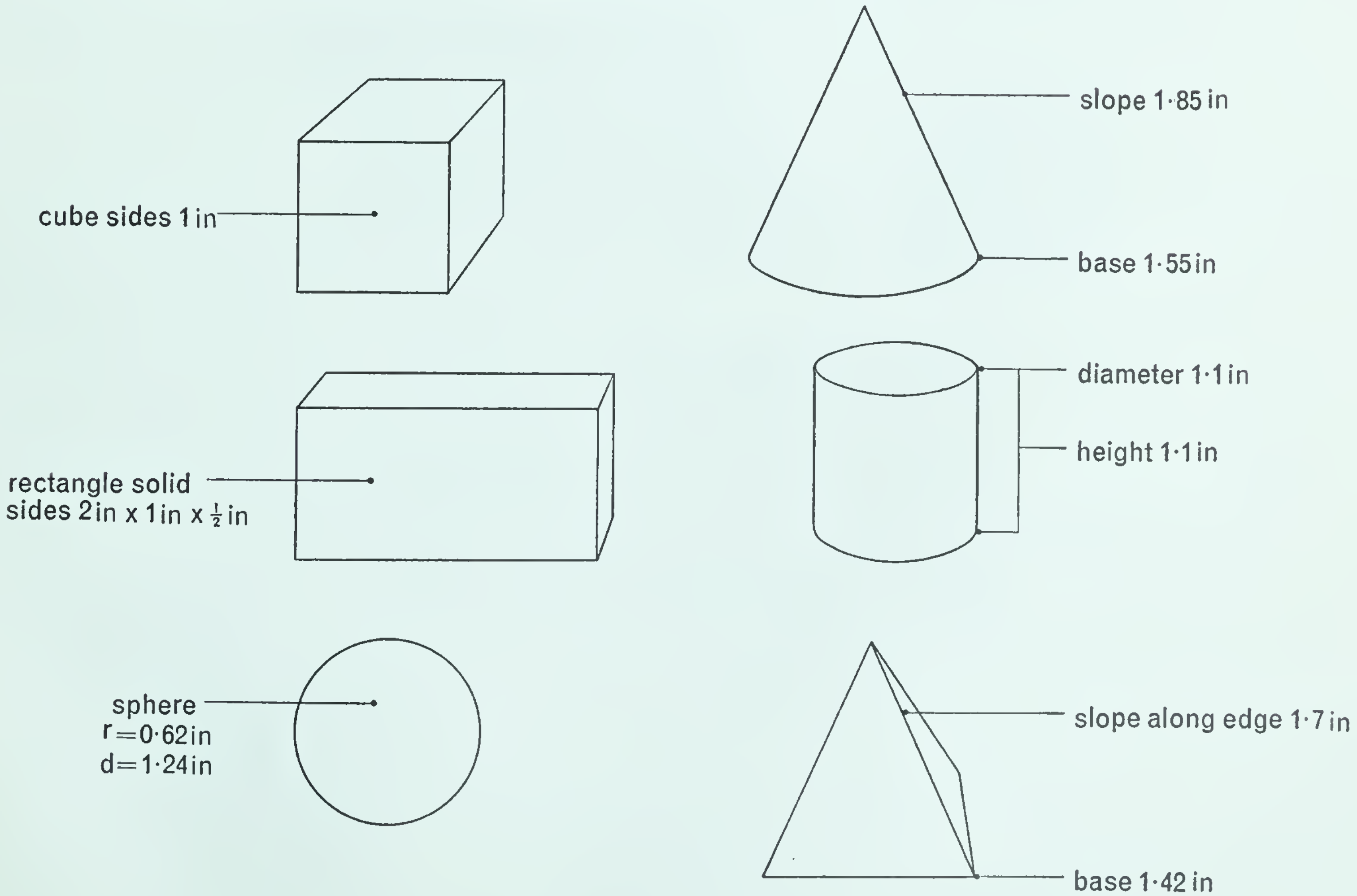
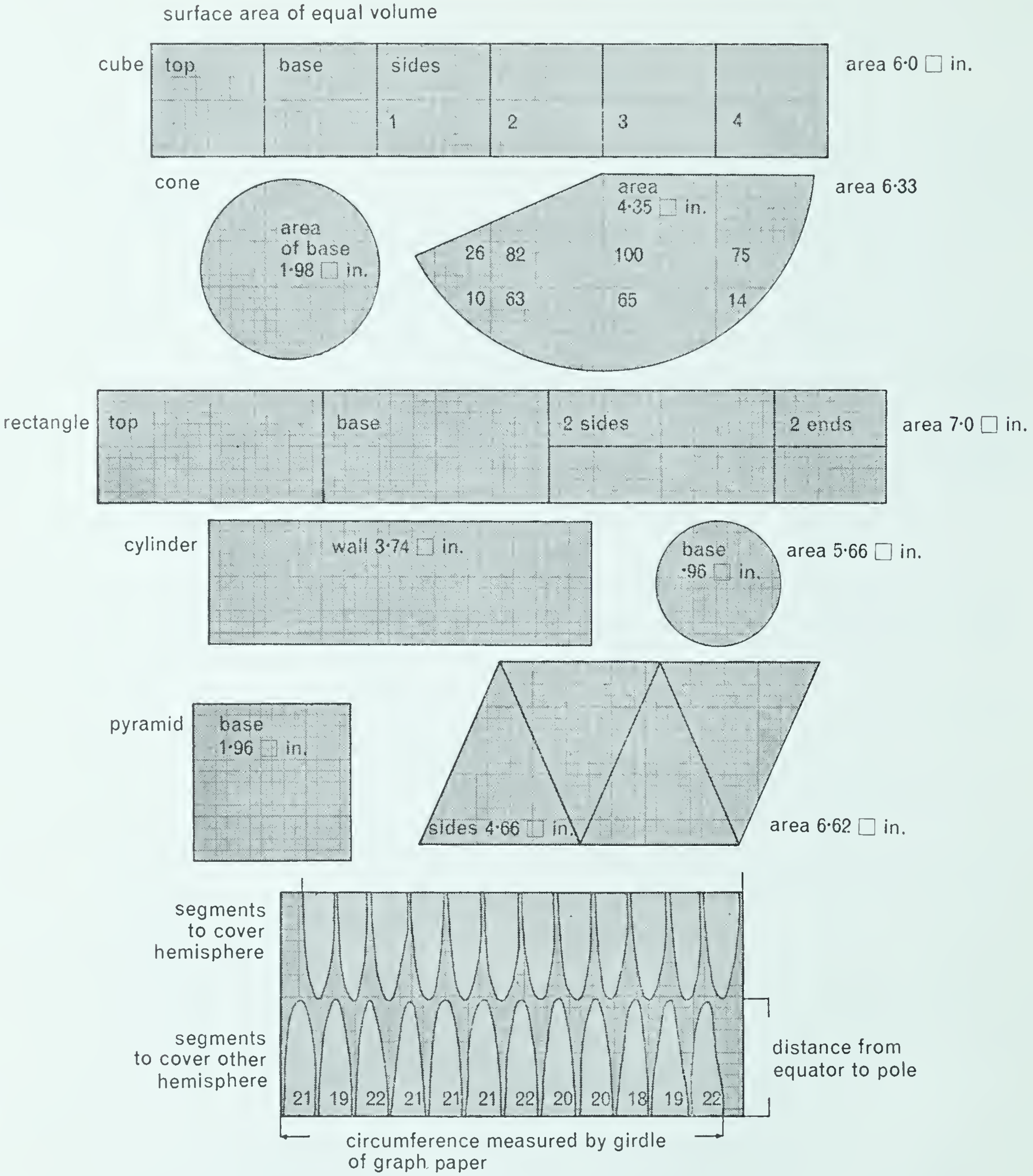


Figure G8.
Drawings and details of equi-volume solids.



sphere of volume
1 cubic inch

circumference as
measured 3·9in.

surface area calculated
$4\pi r^2$ approximately
4·84 □ in.

measured on graph
paper 4·92 □ in.

Figure G9.
Projection of surfaces of equi-volume solids and details of surface areas.

final order
surface areas
in square inches

sphere	4·92
cylinder (squat)	5·66
cube	6·0
cone	6·43
pyramid	6·62
rectangular solid 2in. long	7·0

The units used for various measurements are listed in the text (section 6.1) for reference but pupils should have been using them throughout Chapter 5. The table of dimensions of cubes is also intended for reference during experiments.

Some of these relationships could well be made more real by reference to familiar things. A bubble, for example, has the least surface for its size because surface tension causes the skin to contract to the smallest surface area possible. This also accounts for the rounded shape of many small living things. The carboy is made of the minimum quantity of glass to hold the maximum amount of liquid and the same objective determines the shapes of many other ordinary containers.

The following table shows the mathematical relationships between the various dimensions of certain shapes. As they stand they should be used only by those who wish to treat this subject mathematically.

Formulae for those who wish to treat this subject mathematically:

Shape	*Dimensions*	*Surface area*	*Volume*
Cube	side l	$6l^2$	l^3
Sphere	diameter 2r	$4\pi r^2$	$\frac{4\pi r^3}{3}$
Cylinder	diameter 2r length l	$2\pi r\,(r+l)$	$\pi r^2 l$

The problem may be dealt with graphically, using sets of plastic models of similar volume (1 cubic inch). These are available from mathematical model suppliers, and also from Proops Brothers Limited, 52 Tottenham Court Road, London, W.1.

The models are used as templates to cut out surface pieces in graph paper. By counting the number of squares, the area of the more awkward shapes can be determined. The sphere presents problems but an approximate coverage of paper can be made by using twelve segments to cover each hemisphere (see figures G8 and G9).

6.12
Measuring surface area

Apparatus required

Fully grown mice
Other small animals – e.g. guinea pig or hamster
Sprays of plant material with leaves
Balance weighing to the nearest 0·1 g
Paper for cutting into size for surface area

This will give the children an opportunity to use mice for a simple purpose. Here length of body (excluding tail) will be measured. This may be a good point at which to discuss approximations and to indicate that all parts of a mouse are not as wide as the diameter of the body; therefore the body will have less surface than the model. But on the other hand we have ignored legs, ears, and tail. Should we add an estimate for the surface area of these? Do they more than balance the excess area of the cylinder that represents the body?

If only one mouse is available, this practical can become a demonstration.

To balance the weight of the plant with that of the mouse, cut off the stem and leaves and not just the leaves. Again, there is an approximation involved in measuring the area of leaves by using graph paper and by ignoring the surface area of the stem. The students should see, however, that the (surface/weight) ratio would be little affected.

Again there is an approximation by using rolls or cylinders to represent head, body, arms, and legs. If pupils care to take up the challenge, they could make closely fitting suits of newspaper to cover the whole body as closely as possible, and then find the area. There is a surprisingly close correspondence between the area given by cylinders and that of a more realistic covering.

Pupils should be encouraged to work in metric units as these are usually used in science. A direct comparison can then be made by filling in the chart in the text (section 6.12). The calculation to find the surface area from weight can be tried and checked.

6.2
Surface area and temperature

Notes on teaching

Apparatus required
500 cm³ flasks
100 cm³ flasks
Thermometers in corks to fit flasks
Adhesive
Feathers
Cottonwool

There are a few pitfalls in relating the sizes of animals to heat losses from their surfaces. The argument in the text will have led pupils to the view that for warm-blooded animals living in cold climates, increased size means a smaller ratio of surface to bulk, and therefore a greater economy of heat. For cold-blooded animals, whose body temperatures adjust themselves to the temperature of the environment, however, a small ratio of surface area to bulk may imply a slow response of body temperature to heating or cooling by the environment. This in turn implies that the survival of very large cold-blooded animals may depend on whether they warm up sufficiently, and for long enough, to eat a sufficient supply of food. This means that the sizes of cold-blooded animals may be restricted in such a way that the ratio of surface to bulk is not too small, though much must also depend on other factors, such as the availability of food. Heat loss from the surface also limits the range of smaller warm-blooded animals to warm regions. For at a particular temperature, their metabolic rate must be linked more or less directly to the ratio of surface to volume, and must therefore increase with decreasing size. And the lower the temperature, the greater must be the metabolic rate. This is why the shrew, in its marginal existence in England, is forced to eat for roughly eighteen hours out of every day.

Heat loss from the surface is not the only determinant of size, of course. The availability of food also matters. Thus desert animals are small because the vegetation is sparse on account of competition between plants for water. In tropical areas of lush vegetation, on the other hand, large sizes are once more possible because of the lower metabolic rate associated with increased size and special devices (such as panting) to get rid of heat generated by the muscles.

The relationship between bulk and heat lost or gained from the surface is naturally influenced by the characteristics of the metabolism of certain organisms. Thus fish which live in cold seas and take several years to reach maturity, in spite of the abundance of food available, can survive with a substantially lower metabolic rate than comparable land animals. This means that fish in cold seas grow much larger than the simplest consideration of the temperature of the environment would suggest.

6.21
Heat loss and surface area

Round bottomed flasks should be supported by the neck at about 45° and the bulb completely filled with water. To allow for contraction of the water on cooling, the level should be about 1 inch up the neck. Support the thermometer with the bulb at the centre of the flask.

A much more striking difference is obtained using calorimeters with all dimensions doubled. This gives a four-fold increase in surface for an eight-fold increase in the volume of water.

To avoid confusion between heat and temperature it may be a good thing to use equal volumes of water in any comparisons. Using the flasks, fill one 500 cm^3 with near boiling water to compare it with five 100 cm^3 flasks with the same volume of water shared among them. This means having eight small calorimeters filled with the volume of water that will just fill one larger one. The rate of loss of heat is then clearly indicated by fall in temperature in *equal* masses of water.

Biologically it is the rate of cooling, i.e. drop in temperature, which is important.

There is no need to become involved in measurement of heat at this stage but it is important to avoid confusion between heat and temperature. The measurement of heat and relationship of heat to mass will be dealt with in later work on metabolism.

The relationship between heat loss and surface area can also be well demonstrated by using a series of small tins which together will hold the same volume as one large tin. The cooling of the large tin of water can be compared with individual small tins.

Everyday experience will also help to establish the principle of heat loss through the surface – spreading food out to cool, comparing the cooling of tea in wide shallow cups with that in deeper narrower cups.

6.22
Keeping warm

This section should lead to interesting discussions using the information given in section 6.2 and the results of experiments. When preparing the flasks with feather and cottonwool coverings the adhesive must be allowed to dry out thoroughly so that there is no heat loss due to evaporation of water.

Humming bird

The smallest humming bird is Princess Helen's humming bird of Cuba with a body 2 inches long, bill $\frac{2}{5}$ inch, nest cavity $\frac{3}{4}$ inch and eggs $\frac{3}{10}$ inch long. Its weight is less than 2 g. It cannot survive outside warm regions.

Whales

The whale is a fruitful source of problems. The high insulation of its blubber layer and large size permit it to live in extremely cold seas. It is thought that the fins may act as radiators to channel off the enormous release of heat from its muscles when active.

6.23
Keeping cool

Commonplace examples of ways in which area is extended by folding will be useful here, e.g. ears, wings, fins, etc. The experiment can be presented in class as a demonstration, but it is also very suitable for an investigation at home. Pupils may already be familiar with the fluted filter paper used in chemistry to increase filtering surfaces. Everyday domestic examples of ways to increase surface area are the 'fins' on radiators which give a greater surface for contact with the air and also those on air-cooled cylinders in small engines. Coiled filaments of electric light bulbs increase the glowing surface and therefore the amount of light given out. Pupils may be familiar with the 'bunch of grapes' effect of the alveoli of the lung which increases the internal surface to an area of the order of several tennis courts.

6.3
Retaining water

Notes on teaching

Apparatus required

Sheets of pink blotting paper
Plates of glass
Rulers
A variety of vessels which will hold 10 cm^3 of water to expose different area of surface in each case
Sprays of leaves standing in water

The relationship between water loss and exposed surface can be roughly established as a result of our experience in drying out wet clothing. The handkerchief experiment suggested provides a link between an impression of a relationship and one which can be roughly measured.

Even more precise is the blotting paper experiment where the areas can be measured accurately. This experiment can be more fully quantitative by weighing the pads of wet blotting paper at intervals. Here is an opportunity for pupils to design their own experiments. Dishes and containers are provided so that equal volumes of water can be left on the bench with differing surfaces exposed for evaporation. The comparison between the evaporation of a shallow layer of water in a Petri dish and a deep column with little surface exposed in a test-tube is very striking. This study too, can be made quantitative by weighing the containers at intervals and recording the loss. Each container would, of course, start with the same volume of water. Liquid paraffin provides a good oil layer for the prevention of evaporation.

This series of small experiments gives a good illustration of how the scientist works.

a. Observation – clothes dry slowly when bundled up, and more quickly when spread out.
b. Crude experiment to establish relationships – handkerchief in a ball compared with a similar handkerchief spread out.
c. Hypothesis – statement of supposed relationship, 'the rate of evaporation is proportional to the surface area exposed'.
d. Quantitative test of hypothesis – using blotting paper with an easily defined and measured area and weighing it at intervals to record loss of water.
e. A further series of experiments – to try out the hypothesis. Design an experiment to isolate one factor only – the surface of the water in a container – and compare notes of loss from *equal* volumes of water with different surfaces.
f. Continued experiment – as (*e*) but with the surface of water in one set of containers covered with oil.

At this stage the visual evidence of wilting and loss of weight due to the evaporation of water from large leaves is sufficient. A spray of leaves in a test-tube of water with the water surface covered by oil gives a ready demonstration of water loss. A control tube without leaves should be included. No attempt, at this stage, should be made to introduce the potometer.

6.31
How is water conserved?

As an introduction to conservation of water by animals the drying up of animals with soft bodies and moist skins when exposed to sun or wind can be discussed. Most pupils will have seen dried up slugs, worms, or frogs on footpaths.

No mention has been made of surface tension as we are primarily concerned here with evaporation of water from surfaces and the use of waterproof substances to keep the water in.

Children may be familiar with water-repellent substances as used in proofing cotton and in drip-dry materials. Here water is prevented from soaking in; it is repelled from the pores in the fabric and will not cling to the outside. Thus there is little water left to evaporate. The same principle can be applied to the surface of animals.

Many of these points will be taken up later in the course when water relationships in animals and plants are discussed. Here we are concerned to bring to the notice of pupils some examples of water losses which are due to difference in surface area.

6.32
The advantage of keeping dry

Apparatus required
Blotting paper
Paraffin wax melted in a tin

Wooden or cardboard cubes with felt stuck on all surfaces

Pupils have seen that waterproofing prevents evaporation and so conserves water. In many animals and some plants the opposite effect of penetration and wetting of the surface presents problems.

The weight of water carried by a wet animal becomes significant when the surface is large compared to its bulk – that is in small animals.

Surfaces vary in their capacity to absorb and hold water. There is a conflict here between insulating materials and the weight of water an animal carries when wet. In large animals this is merely unpleasant but because smaller animals have a relatively large surface, the sheer weight of water can become a burden. A wet mouse can only just drag itself along.

Demonstration: How much water is held by easily wetted surfaces

Make cardboard or wooden cubes with sides 1 in, 2 in, and 3 in.

Cover these with thin felt and record the weight of each. Soak each one thoroughly in water and allow to drain. Re-weigh each cube. Then try answering these questions.

What is the ratio of increase in weight to size of cube?

Express the weight of water trapped in the felt as a proportion of the weight when dry.

Does the proportion of water held by the coat increase as the size gets smaller or larger?

Optional additional experiment

Does this experiment cover all the variations of coat with increase in size? Do larger animals tend to have longer coats? As the size of the cube increases try putting twice and three times the thickness of felt. How does this affect the proportion of water held?

Some pupils will by now have realized from these experiments and discussions that two properties are being investigated.

1. *Waterproofing* – the prevention of water passing by an impervious layer. No pores present.
2. *Water-repelling* – this is a surface tension effect when water-repellent substances are in contact with water. It is not intended to introduce surface tension here. The point has been glossed over by referring to substances which repel water or which are not wetted by water.

Confusion arises because many substances, e.g. wax, are both waterproof and water-repellent. Oiled fur, oiled feathers, and the waxed cuticle of insects are good examples of water-repellent surfaces. The use of detergents to make surfaces wet opens up many possible lines for discussion.

6.33
The advantages of keeping wet

The problems facing animals with permanently wet skins are introduced here because they are important in determining where the animals can live. They will be explained further in Years III and IV.

As many of the animals concerned are small, their surface area is large in proportion to their bulk, and water loss is therefore a serious problem. This may necessitate the choice of a humid habitat such as the soil where the surrounding area is usually saturated with water vapour. Alternatively, the animal may create its own micro-climate – such as the frog hopper which lives in a mass of bubbles – cuckoo spit.

A stimulating discussion could well be introduced here on the perils encountered by animals in connection with their surface and size.

The small animal is in danger when on the brink of water. Once wet it cannot escape. A large animal on the other hand, hardly notices the extra weight when wet all over.

References See end of Chapter 5.

References

Chapter 7

Movement in animals and plants

Objectives

The class experiments and discussions in Chapters 5 and 6 will have shown that many of the problems of shape and size are closely related to those of movement. Movement, indeed, is a characteristic of all living things. In this chapter we are concerned with movement and the various structures concerned in bringing it about.

The chapter develops as follows:

1. The movement of the whole organism is observed in a wide variety of animals and compared with the static nature of plants.
2. Movement is then related to the place where animals live and to their mode of life.
3. Particular attention is paid to aquatic animals and to the investigation of movement in fishes.
4. The placing of fins to stabilize movement is important for the correction of roll, pitch, and yaw.
5. The large muscles which drive a fish are exposed in order to show the way in which they act antagonistically.
6. Models to demonstrate the part played by the tail fin are compared with the propulsion of a boat in water.
7. Individual movements are caused by pairs of muscles acting in opposition.
8. The properties of muscles are investigated using elastic models to determine the influence of
a. cross-sectional area
b. length.
9. Force exerted by muscles is related to its cross-sectional area, and the work done to its cross-sectional area and length.
10. Simple movements of plants are observed and recorded.

Suggestions for practical work
Class practical

Section		Page *Text*	*Guide*
7.11	Muscles of a fish	98	71
7.31	Muscles and movement	102	74
7.32	Muscles of the upper arm	104	74
7.4	How does the cross-sectional area of a muscle affect its pull?	105	75

7.41	Is there a change of volume when muscles contract?	106	75
7.42	How does the length of a muscle affect its pull?	108	75
7.5	Observation of movement of petals in tulip flowers	110	76
7.51	Movement of shoot tips during growth	112	76
7.52	Growth movements in roots and shoots of bean	113	77

Demonstrations

7.31	Demonstration model of bones and muscles of arm	102	74
7.5	Sleep movement of plants Demonstration collection of plants which show sleep movements Sensitive plant seedlings Plants kept in a room with lateral lighting to show growth movement in response to light	110	76

Notes on teaching and practical details

Pupils will already have a wide experience of the movement of living things. This should be increased, if possible, by visits to a zoo or an aquarium and a display of a number of small animals in the laboratory. They should observe the use of individual muscles wherever possible, and note the use of limbs as levers.

7.1
Movement in animals

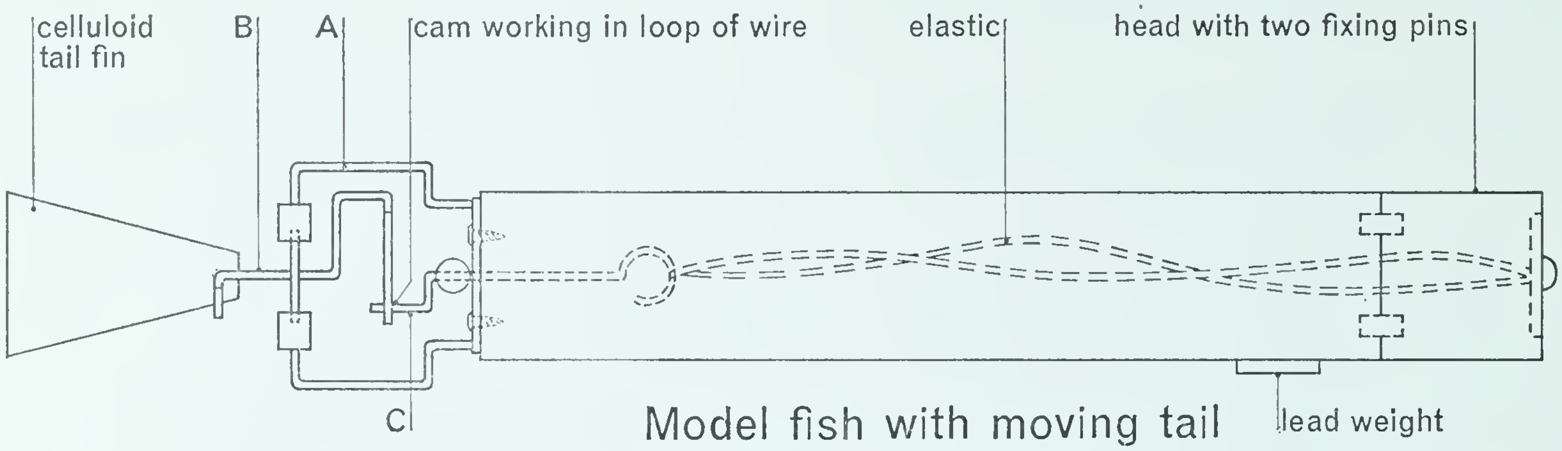
Display as large a collection as possible of living animals including land vertebrates, insects, fish, and aquatic invertebrates. Pictures of animals in movement will also help.

7.11
Muscles and movement in fishes

Apparatus per group of four
Sharp scalpels
Scissors
Fresh whole fish (herring or mackerel) one per group
Dissecting board

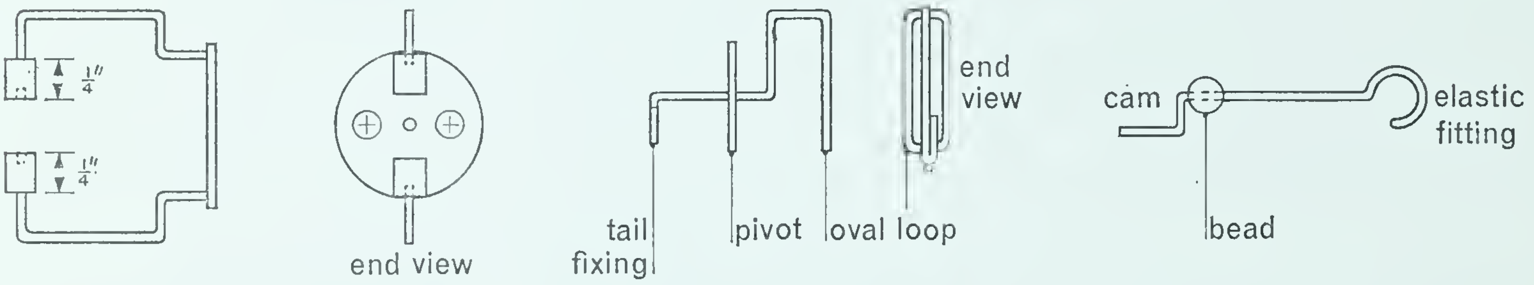
The simple dissection outlined in the text should enable the pupils to see the placing of the powerful muscles which cause the flexing of the body of the fish. Emphasize the opposing groups of muscles on either side of the body so placed that when one side contracts the other side is stretched.

As movement in water has so much influence on structure, it is reasonable to start this chapter with the movement of a fish – an approach which has the added advantage that the basic method of movement is remarkably constant throughout the group. Perhaps the least suitable fish is the common stickleback because of the unusual position of the fins, but goldfish, minnows, carp, and most tropical fish are suitable. Students should be encouraged to look at a wide range of aquatic animals. Frog tadpoles are particularly suitable for observing the S curves of the body which result in



Model fish with moving tail

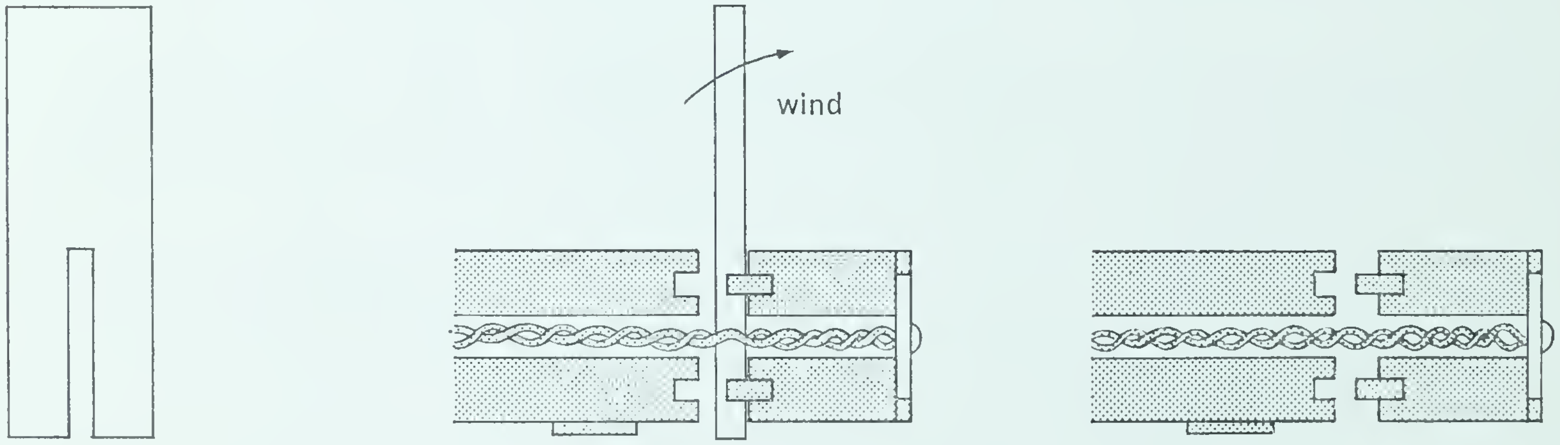
the elastic is wound by pulling the head away from the body and turning the head



A brass plate and bearing for tail unit

B tail unit

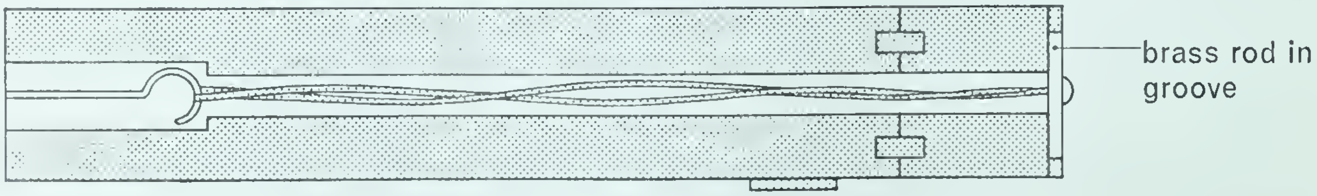
C elastic fixing and cam



D winding handle made in 1/8″ plastic to insert between head

E head and body with Perspex winding handle inserted

head pulled out to winding position



vertical section

Figure G10.
Details of construction of model fish with tail.

forward movement. They are not so well stabilized by fins and therefore yaw to a marked extent.

Undoubtedly the most convincing experiment to show that a side to side movement can be responsible for propelling an object forwards is the use of a single oar as a scull at the rear of a boat. If a boat is available with a central rowlock or slot at the stern this should be demonstrated.

7.12
The use of a tail fin

Apparatus – demonstration model

The model fish described in the students' text can easily be constructed from simple materials available in a school. However, the model, or something similar, is worth making since it demonstrates the action of a tail fin in a most striking manner.

Start with a one-inch dowel or broom handle drilled with a central hole to take a double piece of elastic. At the rear end of the main part of the body, the elastic is fastened to a wire crank using beads as bearings to reduce friction. The wire crank in turn drives the tail from side to side. The material of the tail fin must be flexible. The short front portion of the model to which the elastic is attached is turned to wind up the elastic and is locked to the main body of the fish by two pins when the elastic is fully wound. The design can be modified in many ways, but it is essential that the flexible tail should flap vigorously from side to side. Text figure 54 should give sufficient detail of construction for pupils to show how the model works. Further details for construction are shown in figure G10.

At this stage it is probably not desirable to analyse in detail the forces on the fish tail unless the pupils are already familiar with the necessary physics. This means that you will have to rely on a simple description involving the tail pushing on the water and the water in turn pushing the fish forward.

7.13
Feeling the force of a 'fin' on water

The best experience here is undoubtedly observation of fish from above.

7.2
Land animals and water animals

The section as written in the text should present little difficulty. It is a matter for observation and interpretation of diagrams. It forms the link between the whole body movements of aquatic vertebrates and the limb movements of land vertebrates. These in turn are the basis of individual muscle action to be studied next.

For students who wish to pursue this subject further, there is an easy and fascinating series of observations to be made showing how different modes of movement are adapted to different ways of life. Thus mud skippers and climbing fish exhibit movements which are merely those of a fish 'swimming' on land. Amphibians such as newts have raised the body off the ground and adopted a waddling motion using the limbs with three-point contact with the ground when the animal is on the move. The twist of the limbs to bring them directly underneath the body allows a forward and backward swing and leads to the normal quadruped mode of progression. Variations of this to suit different types of movement – walking, running, trotting, leaping, galloping – are illustrated in well-known animals. Further adaptations to climbing, flight, swimming, and walking on two legs fit into place in the general theme. This can all be done at the level of natural history. The idea of evolution should not be introduced at this stage as it causes confused thinking later on.

7.3
Movement in land animals

The main point to be established in this section is that muscles are arranged in opposing pairs and that any one muscle can do work only by contraction. Lack of physics may again present difficulties when the bone is dealt with as a machine which changes the direction of a force. No attempt has been made at this stage to introduce mechanical advantage or velocity ratio. The bone is a simple lever which turns about a pivot and so converts a pull into a push.

7.31
Muscles and movement in land animals

Apparatus required
Bricks or heavy weights for each pair of pupils
Shelf or stool on the bench at shoulder height

It is difficult to feel which muscle does work in an action. We become consciously aware of using a muscle only when it begins to tire and ache. The experiment suggested to the pupils relies on this. To isolate the action of one muscle it is best to have the pupils sitting alongside a shelf, or other temporary support for the bricks, at shoulder height. Two bricks are sufficient if pupils work in pairs. The assistant takes each brick in turn from the fully stretched arm of the experimenter and replaces it on the shelf at shoulder level. The triceps muscle soon begins to ache.

7.32
Muscles of the upper arm

Apparatus required
Demonstration model of articulated skeleton of the arm with rubber tubing model muscles (see text figure 58)
Balsa wood for pupils to make models of arm bones

By far the most satisfactory model is an articulated skeleton of the arm with muscles made of bicycle inner tubing. Leave the tube intact in the middle section to represent the muscle. Cut it longitudinally at the upper end and stick each half round the Bunsen tubing to form the two tendons which attach to the scapula. Quick setting contact adhesives give satisfactory results. Cut out four or five V-shaped pieces from the lower end and taper the muscle down by sticking the flaps to Bunsen tubing to form the tendon which will be stuck to the radius. Deal with the triceps in a similar way, making three tendons to attach to the humerus and the scapula at the upper end and one to attach to the ulna at the lower end. The model can be made very realistic by including in each muscle tube a balloon firmly attached to plastic tubing. When you inflate the balloon the muscle contracts. (Care should be taken to point out that this is not how muscles normally work.)

Less realistic models of bones can be made using balsa wood, hardboard, Meccano strips, or strong card.

7.4
Cross-sectional area and the pull of muscles

Apparatus per group of four
Lengths of $\frac{1}{4}$ inch, $\frac{3}{16}$ inch, and $\frac{1}{8}$ inch catapult elastic (square section aircraft elastic)
Spring balances reading 0–100 g and 0–1,000 g
Retort stands and clamps

When using elastic to represent muscle it is essential to point out that the spring balance is stretching the elastic but that the elastic in turn is exerting a pull on the balance. This is equivalent to the pull of a muscle when it contracts and is the amount registered on the spring. It is much easier to use a spring, and not weights, to extend the elastic. The term pull has been purposely used instead of forces as students will be more familiar with it unless they have been following the Nuffield Physics course.

The details of the apparatus should be clear from text figure 59. It is probably advisable to use a 100 g balance for the $\frac{1}{8}$ inch elastic to obtain a reading of the pull to the nearest g. To save time each length of elastic should be tied at points near the end to leave a gap between the ties of 20 cm when just pulled straight. Most pupils will be able to read off the extension using the top string as a marker. To avoid parallax, a pin can be pushed through the top knot and this serves as a pointer on the scale when the elastic is stretched.

Although square section elastic is sold in $\frac{1}{4}$ inch, $\frac{3}{16}$ inch, and $\frac{1}{8}$ inch thickness and we refer to these sizes, all measurements are made in centimetres and millimetres.

7.41
Muscle contraction and change of volume

Apparatus per group of four
Length of $\frac{1}{4}$ inch square catapult elastic
Pins
Suitable width and length gauge

It is difficult to demonstrate that the volume of a muscle remains constant when it contracts. Perhaps the simplest way is to use catapult elastic. Nominal $\frac{1}{4}$ inch square elastic is about 0·6 cm square before use. Mark a 10 cm length on a piece about 20 cm long using pins pushed through the elastic. The volume of the marked piece will be $10 \times 0{\cdot}6 \times 0{\cdot}6\ \text{cm}^3 = 3{\cdot}6\ \text{cm}^3$. Place, across the ruler, a micrometer gauge with the gap set at 0·5 cm, and pull out the elastic until it just fits the gap in the gauge. Read off the length between the two pins on the ruler. Repeat with the micrometer set at 0·4 cm.

If a micrometer is not available grooves filed in a sheet of tin or Perspex can serve as a measuring gauge (see text figure 60).

7.42
Length and pull of a muscle

There is no difficulty in demonstrating that pull is constant for a fixed proportional extension no matter how long the elastic.

Pupils should arrive at this conclusion from their experiment. They may need a little convincing that what applies to elastic is also true of muscle, or that the pull of a muscle is independent of length. At this point it must be made clear that pull (or force) and work are very different. It may be necessary to use homely examples such as pulling a garden roller by a length of rope. The pull remains the same no matter how much rope is used. What is different is the total amount of work you have to do when you haul in the roller on 10 ft of rope compared with hauling in 20 ft of rope – you apply the pull for twice as long. If more work is done in a given time, greater power is used.

7.5
Sleep movement of plants

Notes on teaching

Apparatus required

Any of the following plants:
Sensitive plant (*Mimosa pudica*) or Venus' fly trap
Tulips in close bud
Clover
Evening primrose

Microscopic plants which show movement have been purposely omitted. Moreover, the fundamental movement within protoplasm in both plants and animals is not our concern here either. The theme of the chapter so far has been movement by muscles. In this context plants have been regarded as non-movers as they stay in one place. This also fits in with previous chapters on size and shape which were also related to non-movement in plants. But it will be obvious to pupils that parts of plants do, in fact, move, so that some of these movements should be studied. The movement of parts of plants is divided roughly into the comparatively rapid ones (for plants) such as sleep movements, the opening and closing of flowers, and the movements of sensitive plants and Venus' fly trap, as well as the slower turning movements due to growth. No explanation of the mechanism is offered in either case.

Opening and closing of flowers

As this work is scheduled for the spring term the tulip provides a ready source. Care must be taken to obtain young tulips in the bud stage, just opening. Tulips will normally only open and close three or four times. If a closed flower which has already opened and closed three times is used there will be no reversal after the final opening.

Seedlings of *Mimosa pudica* can be raised early in the year in a heated greenhouse. The seeds are sold by most seedsmen. Sensitivity is shown by the first pinnate leaf to emerge from the cotyledons. A convenient way of growing the plant for class use is in lettuce tubes or minipots, packed forty to a seed box and filled with John Innes Potting Compost No. 2. Sow a single seed in each tube. Pupils are most interested in this plant and a lettuce tube with a plant can be taken home and grown in the house in a pot.

The Venus' fly trap has recently been popularized as a house plant and supplies are available from seedsmen.

7.51
Movement of shoot tips during growth

Apparatus per group of four
Runner bean seedlings at about four leaf stage (three to four weeks after sowing)
Glass plate 5 × 5 in
Cardboard with pinhole
Retort stand and clamps

The runner beans for this experiment should be planted one month before they are required by the class. Germination is usually almost 100 per cent, so only one seed need be sown in each $2\frac{1}{2}$ inch (size 60) pot. Clay or plastic pots are equally suitable and the bean is best grown in John Innes Potting Compost No. 2. One seedling for each group of four pupils is sufficient as there are numerous recordings to be made in which the group can share. The experiment needs a greenhouse with all round lighting, or phototropism will alter the pattern of growth of the shoot tip. If a greenhouse is not available the experiment should be conducted in a room and shielded from the direct light of the windows. Overhead illumination can then be provided by a 100 W electric light bulb about 2 ft above the plant.

The main source of error in this experiment is movement of the plant or its pot. Clamping the pot to a retort stand base by two wide elastic bands passing over the pot and under the base is the easiest method of securing it. Movement of the plant can be prevented to a large extent by two or three ties to a short cane.

Marking the position of the shoot at intervals throughout the growing period is best done using a normal ink pen on the glass plate or, more distinctly, by waterproof Indian ink. The general phenomenon of circumnutation in plants can be followed up by suggesting further experiments on other plants. Pupils can be encouraged to examine climbing plants and to make lists of clockwise and anti-clockwise twiners. A useful garden project is to have a special section for climbers in which to test their ability to climb supports of various thickness from string to a tree trunk. A further experiment on the angle of climbing can be devised using canes set at different angles in the ground in 10° steps from horizontal to vertical.

7.52
Growth movements in a broad bean plant

Apparatus per group of four
Sheets of glass 6 × 5 in
$\frac{3}{8}$ inch and $\frac{1}{2}$ inch square section wood
Damp peat
Broad bean seeds

It is not the intention at this stage to investigate tropisms in plants, but to establish the fact that tropic movements do occur. Pupils should already be aware of this change in direction of growth through the observations they have made when plants in the garden have been blown over. As the 'germination sandwich' has already been used in Year I, Chapter 4, it is convenient to bring it in here as an easy means of demonstrating geotropism in the root.

'Germination sandwiches' can be made in a variety of forms. The essential feature is that a layer of damp material is held between two glass plates spaced at the correct distance just to accommodate the swollen seed being investigated. Wooden spacing strips of square section are placed around the edge of the sandwich, which serve to hold the plates apart and to retain the moist material. Peat, sawdust, or vermiculite are equally suitable provided they are well soaked. The glass plates can be held together by broad rubber bands or Sellotape.

Balsa wood strips or cedar strips can be obtained from model suppliers. Square section $\frac{1}{2}$–$\frac{3}{8}$ inch will be sufficient for all needs. Sheets of glass, all the same size and roughly 6 inches by 5 inches can be cut from horticultural glass or obtained cut to size from most builders' suppliers.

The germination sandwich can be suspended from a hook at eye level on a shelf or wall. Growth is best if the front is covered between observations by a flap of black paper.

Class sets can be placed for storage in blocks on shelves as if they were books, but they must always be stored the right way up.

If the top spacer bar is left loose by arranging the binding bands horizontally around the sandwich, then seeds can be added at intervals and seedlings withdrawn for marking when necessary.

References See Chapter 5.

Chapter 8

How plants reproduce and make seeds

Objectives

In this chapter it is shown that sexual reproduction in plants follows the same pattern as in animals, involving the production of pollen by the male part of the flower and ovules by the female part of the flower. Fertilization in plants is the union of a male gamete with a female gamete. Some of the mechanisms used by plants to ensure cross-pollination and self-pollination are investigated and it is proved that fertilization of the ovule results in the production of a seed. Seeds grow inside the ovary and when ripe, they germinate to produce new plants.

The order of development of the chapter is as follows:

1. A flower consists of certain essential parts.
2. Each of these has specific functions.
3. The anthers produce pollen.
4. Different flowers produce pollen of different colour, shape, and size.
5. Pollen is carried from one flower to another by insects and by wind.
6. Pollen grains germinate to form a tube which grows down to the ovary.
7. Fertilization means that the pollen nucleus unites with that of the ovule.
8. Fertilization results in the formation of a seed.
9. Some flowers are self-pollinated; others have various devices to ensure cross-pollination.
10. Seeds ripen within the fruit.
11. If seeds are sown they grow into a new plant that will, in turn, produce flowers and set seed.

Suggestions for practical work

Class practical work

		Page	
Section		*Text*	*Guide*
8.11	Finding the different parts of a flower	117	81
8.14	Examining different kinds of pollen	120	81
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8.18	Growing pollen grains	127	87
8.2	What happens if pollen is not allowed to reach the stigma?	134	91

	8.3	The fruit ripens	137	93
	8.32	Sowing ripe seeds	139	93
Demonstrations	8.14	Prepared slide of different kinds of pollen grains	120	81
	8.16	Prepared slide of the third leg of *Apis mellifera*	124	83
	8.19	Prepared slide of pollen grains germinating on a stigma of *Oenothera lamarckiana*	133	90
Optional practical work	8.12	Examining the bud of a flower	119	81
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	8.21	Self-pollination is possible	135	92

Planning ahead

This chapter is intended to be taught at the beginning of the summer term when all the plant material suggested should be readily available. Alternatives are suggested for each section.

The emasculation experiments to show that pollen is necessary for fertilization of the ovules (section 8.2) radish plants (or any crucifer such as mustard) are suggested. These are required in flower. Seeds sown during the first week of term should be ready in three to four weeks, and the results of the emasculation experiments can be recorded later on in the term.

It may be helpful to list the plant material suggested for this chapter. Alternatives are given in the teaching notes to each section:

8.11 and 8.12 Single-flowered Brompton stock
8.14 Tulip, hyacinth, bluebell, primrose, dandelion, alder, or pussy willow flowers
8.15 Buttercup, columbine, honeysuckle flowers
8.16 A variety of flowers growing wild or in the garden which can be observed for visiting insects
8.17 False oat grass (*Arrhenatherum elatius*), rye grass (*Lolium perenne*)
8.18 Narcissus, tulip, bluebell flowers, common chickweed (*Stellaria media*)
8.2 Radish in flower
8.21 Tulip flowers
8.22 White deadnettle (*Lamium album*), primrose (*Primula vulgaris*), wild arum (*Arum maculatum*), sage (*Salvia pratensis*) as material for examination
8.3 Apple fruits in different stages of development
8.31 Pea pods, poppy fruit, antirrhinum fruit
8.32 Packet of seeds of the following: pea, lupin, nasturtium, sweet pea, dandelion, groundsel, shepherd's purse

8.11
Finding the different parts of a flower

Apparatus required per pair

Single-flowered spike of Brompton stock with some flowers fully open
Hand lens × 8
Mounted needle
Scalpel
Watch-glass

Notes on teaching

A wallflower has been illustrated in text figure 63. To avoid direct copying, it is better to provide the class with another crucifer such as Brompton stock, which should be the single-flowered variety. Any large-flowered crucifer will do equally well.

If time permits, the examination of more than one kind of flower would allow the differences in the various parts to be appreciated. The structure and shape of the stigma, length of style, number of stamens, shape of anthers, and position of nectaries could then be compared. Selecting one of the *Rosaceae* would offer an opportunity for comparing a hypogynous form (wallflower) with a perigynous form (*Rosaceae*) – a useful comparison when it comes to the formation of the fruit in the apple (section 8.3).

It is important that the class should find the ovules within the ovary and see the pollen grains on an anther. These may not be visible through a × 8 hand lens and may have to be demonstrated under the low power of a microscope. It should be pointed out that the state of the stamens will, of course, vary from one flower to another.

8.12
Examining the bud of a flower (optional)

Apparatus required – as for section 8.11

Notes on teaching

Select a fairly tight bud in order to ensure that the anthers have not dehisced (burst open) and that the ovules are still immature.

8.13
The anthers and pollen ripen (optional)

Apparatus required per pair

1 tulip or lily
Mounted needle
× 8 hand lens

Notes on teaching

So far the intact anther has been examined. By cutting a large unripe anther transversely, the pollen within the pollen sacs can be seen and, if possible, a ripe anther can also be examined to show dehiscence. Compare both with text figure 64. If suitable flowers are not available in quantity, this investigation can be a demonstration.

8.14
Examining different kinds of pollen

Apparatus required per group of four or six

Microscope slides and cover-slips
Dissecting microscope
Selection of flowers such as tulip, hyacinth, bluebell, primrose, dandelion, alder, or pussy willow

Notes on teaching

In this practical we are concerned only with the fact that pollen grains vary in size and colour in different flowers. This should be apparent under low power. As many different flowers as possible should be provided.

Under high power the external structure of the grains is easily visible and the fact that some grains are smooth, some have prickly walls, while others have air sacs, one on each side, is not accounted for until the different agencies of pollination are discussed in sections 8.16 and 8.17. Permanent preparations of different types of pollen can be obtained from biological suppliers. A temporary mount of various pollens can easily be made and projected on a microprojector. Dark background illumination shows up the grains.

Pollen

Pollen grains vary enormously in shape, size, and in other characteristics. They may range in size from 0·0025 mm in diameter (*Myosotis*) to 0·25 mm in diameter (*Cucurbita*). The wall of the grain consists of an outer layer, the extine, and a thin inner layer, the intine. The extine often has thin areas through which the pollen tube, an extension of the intine, makes its way. It is interesting to note that in plants with water-borne pollen the extine is usually absent.

In plants pollinated by insects, the extine is often sculptured in various ways, bearing warts, ridges, spines or bristles so that the grains tend to stick together and become attached to the hairy legs of the insects (see text figure 70a). In wind-pollinated plants the grains usually have an airfilled sac on either side which not only increases their surface area but makes the grain lighter. This is seen to perfection in the pollen of *Pinus* (see text figure 70b).

8.15

Insects and flowers

Notes on teaching

There is opportunity here for various interpretations of the practical work which follows in this and the succeeding sections. What kinds of insects visit what kinds of flowers? Are there some flowers which are habitually visited by the same species of insect? How are the flowers adapted to encourage pollination by certain insects? The main object of the section is, however, to establish that insects play a very important role in the transference of pollen from one flower to another and that while the flower gains in this way from insect visitors, the insects benefit by obtaining nectar and pollen. The various ways in which the design of some flowers ensures cross-pollination are dealt with in section 8.22.

Buttercup, columbine, or honeysuckle are suggested as flowers in which the nectaries are variously situated. Others could be substituted to illustrate the shallow open type of flower in which the nectary is easily accessible to short-tongued crawling insects, the deeper flower with a short spur, and the type of flower with a very long spur at the end of which the nectar is secreted. The latter type are usually visited by moths with a very long proboscis.

Colour and scent of flowers

In addition to the shape of a flower which may be adapted to a particular group of insects, there are also the further selective agencies of colour and scent. There is no doubt that, although bees cannot distinguish between certain colours such as red and green, they are able to distinguish between different colour intensities and that they are definitely attracted by blues and purples. Von Frisch (1919) is at pains to emphasize that when we say these colours attract the bee, we do not mean that the bee 'likes' them but that it comes to associate these colours with the presence of nectar.

On the whole, the flowers which depend upon night-flying moths and other insects for pollination are pale in colour. This group includes such flowers as the evening primrose, honeysuckle, tobacco, and night-scented stock.

By contrast with the fact that flowers which attract hymenopterous insects are usually brightly coloured, those which rely upon wind pollination are usually of a uniform green or pale brown, the flowers being small and insignificant yet adapted to shed masses of pollen onto stigmas which are usually very prominent.

The scent of a flower is, of course, associated with the presence of nectar and, as has been pointed out in the text, not all brightly coloured insect-pollinated flowers produce nectar. The tulip is an example.

Do those flowers which produce nectar do so all the time? It is fairly clear that they do not. One of the things worth some expenditure of time would be to discover which flowers produce nectar for only limited periods of the day. Is production of nectar affected by humidity, temperature, and the age of the flower? Undoubtedly all these points are closely correlated with the needs of the flower to be pollinated. On a wet day, for instance, most flowers remain closed and so prevent the pollen from becoming wet. Under such circumstances a flower which normally produces nectar in the middle of the day when the sun is fully out may have no scent at all. Insects would probably be absent in any case. Immediately after a heavy shower of rain, however, the scents of flowers are often very pronounced.

8.16
Watching insects visiting flowers
Notes on teaching

A great deal has been written about bees and flowers and the fact that insects carry pollen from one flower to another. Nevertheless, much can be learnt by actually watching insects as they visit flowers. How do they alight on a flower? Are they collecting nectar or pollen or possibly both? Thus only pollen is collected from such flowers as tulip and poppy. Other flowers such as buttercups offer nectar as a subsidiary bribe. On many bisexual plants such as pussy willow (*Salix caprea*), the female flowers produce nectar while the male flowers produce pollen (and possibly nectar as well).

But do insects visit the same kind of flower in a single foray?

Questions may also arise about the structure of the insects themselves, which may lead to a closer examination of mouthparts, special structures for carrying pollen, and so forth.

The completion of the table will call for accurate observations which may be best carried out at home. Results could then be compared in class. The greater the variety of flowers used for these observations the better, for this will lead to a thorough knowledge of the diversity of insect and flower interrelationships.

A microscope preparation of the third leg of a honeybee (*Apis mellifera*), projected on a microprojector, would help to explain text figure 68.

Entomophilous flowers

Flowers visited by insects can be divided into a number of classes:

1. *Pollen flowers* – those without nectar but with a superabundance of pollen which is gathered as food, especially by bees. Poppy, rose, and rock rose are examples.
2. *Flowers with exposed nectar* – such as the *Umbelliferae*, maples, lime, and elder. Flowers are usually small, wide open, and visited by short-tongued insects such as flies and beetles, although lime is much visited by hive bees early in the morning when the nectar is flowing.
3. *Flowers with partly concealed nectaries* – such as many of the *Cruciferae* buttercups and willow. The nectaries are partly hidden in corolla tubes by hairs or scales and can be easily reached by short tongued bees and by flies.
4. *Flowers with fully concealed nectaries* – such as the *Compositae*, the heaths, *Myosotis*, thyme, and mint. In these flowers the nectaries lie at the bottom of fairly long corolla tubes and are accessible only to long tongued insects such as bees, hover flies, and moths.
5. *Hymenopterous flowers* – are those visited exclusively by bees and their structure is specialized to permit visits of only one kind of insect or by insects of a limited group. Red clover is an example where the nectar is 9 mm from the mouth of the flower and available only to the bumble bee. Elsewhere it may require a heavy insect to open the flower. The antirrhinum and broom both have a very narrow opening to the deep corolla tube at the base of which is the nectary. Such a flower can only be opened by the bumble bee, although pirate beetles often chew their way through the corolla tube, so reaching the nectar by a short cut. Many zygomorphic flowers such as sage and monkshood have a special landing stage for the bee, and nectaries concealed in a deep corolla tube or in long spurs.
6. *Lepidopterous flowers* – visited almost exclusively by moths and butterflies with nectaries at the base of long spurs. The flowers are usually lighter and without landing stages since the moth or butterfly hovers in front of the flower while sipping the nectar. The honeysuckles belong to this group as well as those flowers pollinated by night-flying moths – the night-scented stock, Nottingham catchfly, and many of the exotic tropical orchids. In such flowers the nectar flows for a limited period in the evening.
7. *Dipterous flowers* – such as the speedwells, are visited chiefly by flies of different kinds and are usually less highly specialized. Some, however, show great specialization, and to this group we could assign the wild arum, an example of a peculiar method of pollination.

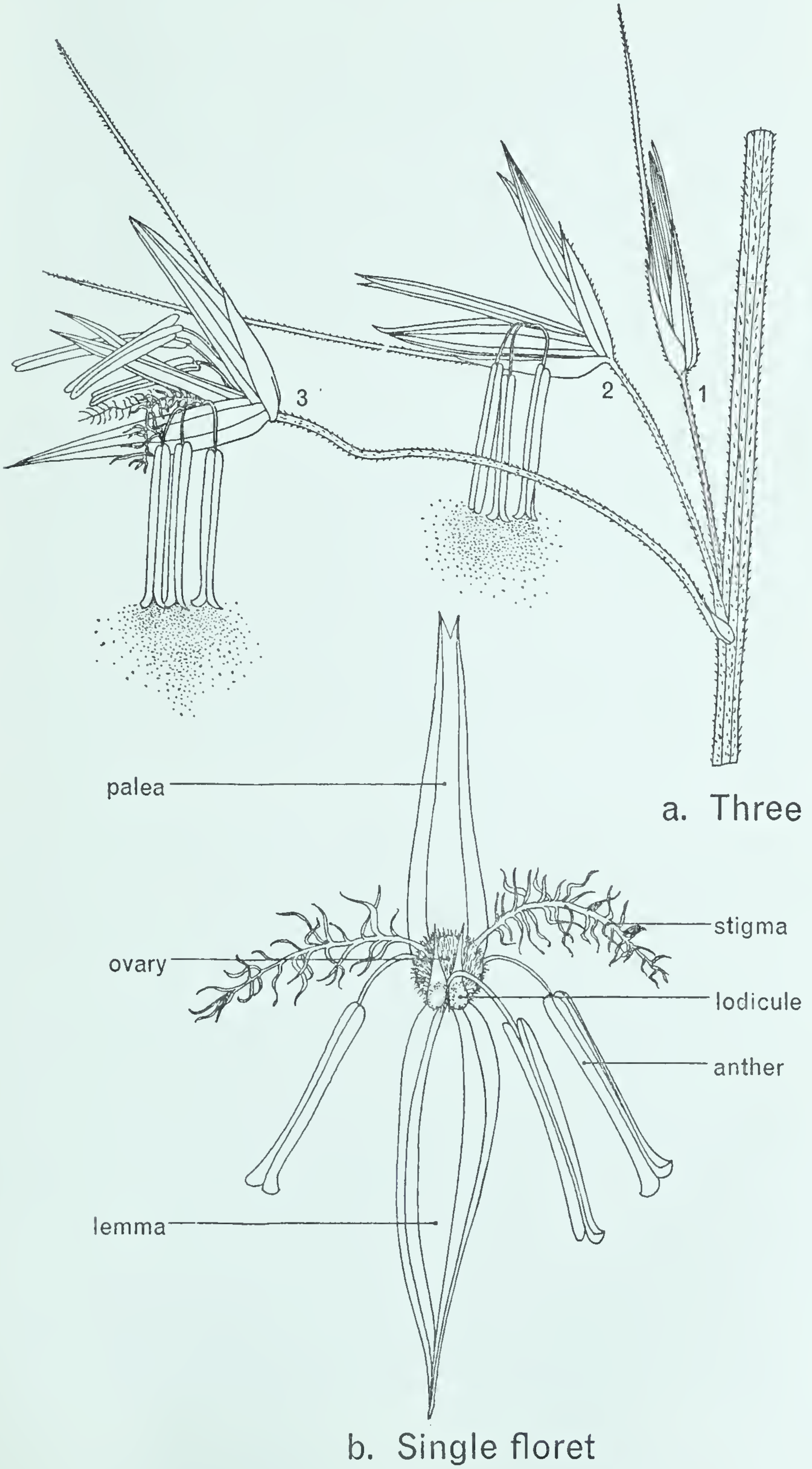


Figure G11.
False oat grass (*Arrhenatherum. elatius*).

8.17

Pollination by wind (optional)

Apparatus required per pair

Mounted needle

Hand lens × 8

Notes on teaching

Probably the easiest wind-pollinated flower to examine will be one of the grasses, which are always available in the early summer months. But many grasses form tight spikelets and it is difficult to dissect a single floret. False oat grass (*Arrhenatherum elatius*) has been suggested since the spikelets are large and the panicles open. But the common oat (*Avena sativa*), rye grass (*Lolium perenne*), or any of the plantains would do equally well. In all these the characteristics of the typically wind-pollinated flower are evident – large anthers on dangling filaments producing masses of light pollen.

Dissection of false oat

Figure G11 shows the panicle of false oat with three spikelets enlarged. Each spikelet may contain two or three flowers. These should be separated with a needle and one flower detached. The drawing (*b*) in figure G11 shows a single floret consisting of three stamens, a round hairy body, the ovary, at the base of which are two small scales, the lodicules. The ovary bears two feathery stigmas on short styles.

After the whole panicle of the oat grass has opened, the filaments of the anthers elongate rapidly causing the three anthers to be pushed over the sides to hang from the floret. At the same time the stigmas spread and project on each side of the open floret.

Notes on wind-pollinated plants

Pollen grains are usually small and quite smooth, and are produced in enormous quantity. Separation of the grains is complete and because of the absence of any sculpturing there is no tendency to stick together. Many types of wind-borne pollen grains have air-filled wings on either side (see text figure 70) which assist in keeping the grain floating in the air.

The attachment of the anthers to the filaments is very delicate, so that the faintest breath of wind sets them shaking. The stigmas are freely exposed and are often long and feathery. In many trees and shrubs which are pollinated by wind, access of pollen to the stigmas is assisted by early flowering so that the leaves, not yet out, offer no obstruction. In grasses this arrangement is not necessary because of the linear foliage, the flower spikes being usually higher than the leaves.

In discussing wind pollination, it should be mentioned that some anemophilous trees such as the elm have hermaphrodite flowers; that some like the hazel and alder have separate male and female flowers on the same tree (monoecious).

Hydrophilous flowers

There are a few examples of water-pollinated flowers. In a great many aquatic species the inflorescence stands above water level and is pollinated by insects (e.g. water lilies, water plantain, and arrowhead) but in a few the pollen is carried from the stamens to stigma by floating on the water. In the case of the Canadian pondweed (*Elodea canadensis*) the whole of the male flower, which is produced singly underwater, is detached and floats up to the surface where it bursts to scatter pollen on the surface. The female flowers are produced at the surface. It should be added that male flowers are extremely rare and that the plant normally reproduces vegetatively.

8.18
Growing pollen grains

Apparatus required per group of four or six
Narcissus, tulip, bluebell
1 Petri dish and filter paper
1 pipette
Visking dialysis tubing (2 inch length)
2 pairs of forceps
1 pair of sharp-pointed scissors
Chinagraph pencil
Test-tube of distilled water
Test-tube of sucrose solution (5 per cent, 10 per cent, or 15 per cent) or of distilled water
Binocular dissecting microscope

Group I	Test-tube of distilled water
Group II	Test-tube of 5 per cent sucrose solution
Group III	Test-tube of 10 per cent sucrose solution
Group IV	Test-tube of 15 per cent sucrose solution

Notes on teaching

The question raised is the means by which the pollen, once stuck to the stigma, reaches the ovary which in some flowers may be some distance away. It may be as well to allow time for a short revision of flower structure here, to emphasize that the ovules lie *inside* the ovary, which is at the base of the style. Thus the pollen must travel down inside the tissue of the style by some means or other. The practical work which follows is designed to show what happens after the pollen falls on the stigma, and we are not concerned here with the actual process of fertilization which will be discussed in the next section.

The experiments designed to show the conditions necessary for pollen germination reveal that different types of pollen require different concentrations of sugar. Thus Bold (1960) quotes successful germination of wild hyacinth pollen in 5 per cent cane sugar while pollen of *Echeveria retusa* will only germinate in a 15 per cent solution of sugar. Brook (1964) states that pollen of *Tradescantia virginiana* will germinate satisfactorily in a 5 per cent sucrose solution in two hours. MacGregor Skene (1924) says that the pollen of primrose germinates equally well in water or sugar solution.

However, it has been known for some time that there are many cases in which germination of pollen under artificial conditions does not readily take place. It was suspected that some essential substance was secreted by the stigma. This was confirmed by placing fragments of stigma in the sugar solution with the pollen. The pollen tubes tended to grow in the direction of the fragments. This could be tried as an alternative practical (see below).

More recently it has been found, under laboratory conditions, that traces of boron are necessary for the successful germination of a number of different pollens (O'Kelly, 1959). The anther of a flower sometimes has a low boron content while the style and stigma may be well supplied. This may be an adaptation to ensure the development of pollen tubes in the right place and at the right time for fertilization, but the issue has not yet been fully investigated.

It seems clear from experiments that different kinds of pollen require different concentrations of sucrose. While in narcissus an 80 per cent germination can be expected by using a 15 per cent solution of sucrose, this is probably too strong for hyacinth or tulip.

We suggest that the class be divided into four groups, as described in the text. Each group should be provided with the same three flowers. Narcissus, tulip, and bluebell, are suggested, although hyacinth or primrose would do as well. It will probably be impossible to make an accurate count of all germinated pollen grains, so a more general estimate can be made. Results should be compared on the blackboard. Visking dialysis tubing is recommended rather than Cellophane, which tends to curl.

Sucrose is used for the sugar solution and made up in the strengths indicated. The length of time that different kinds of pollen take to germinate may vary according to temperature and also to the strength of the sucrose solution. It is, therefore, a good thing to examine the dishes after twenty-four hours to see if germination has gone far enough, and to ensure that mould is not starting to grow. If results are already evident, growth of mould can be arrested by placing the Petri dishes in a refrigerator until the next class practical. Refrigeration will, of course, also prevent germination of the pollen, so that it may be difficult to decide when the dishes should be refrigerated. However, results will be comparable if germination in all of them is allowed to proceed for one day or two days.

Alternative practical

1. Does boron help some kinds of pollen to germinate?

It has been mentioned (in the notes on teaching to section 8.18) that, for some plants, traces of boron appear to be necessary for pollen germination. This offers possibilities for further experimentation, and with a little organization of the practical work suggested for section 8.18 could be included by providing a duplicate set of Petri dishes of sucrose solution or distilled water, adding a trace of boron to each (see below). The results of adding boron can then be compared with those dishes in which there was no boron.

Making up sucrose and boron solutions

Boric acid is a convenient source of boron. Since the pollen grains are placed on Visking dialysis tubing, and do not come into direct contact with the boron and sucrose solution, the concentration of boron is higher than the 2 to 20 parts per million used by O'Kelly. A concentration of 500 parts per million would probably not be too great. To such a solution of boric acid in distilled water is added sucrose to give the 15 per cent, 10 per cent, and 5 per cent solutions.

Alternative practical

2. Germinating pollen grains using fragments of ovary and stigma

Set up the Petri dishes containing the sugar and boron solutions in the same way as in the alternative practical (*1*) using a 5 per cent solution of cane sugar in distilled water. Dust the surface of a piece of Visking dialysis tubing (about 4 cm square) with bluebell (or hyacinth) pollen. Place on the square a fragment of ovary and a fragment of stigma tissue from the bluebell flower. Replace the lid of the Petri dish and incubate at room temperature. Examine after a day or so. Have the pollen tubes grown in the direction of the ovary or stigma fragment?

This experiment, although not a proof of the presence of boron in the stigma and ovary, may show that both stigma and ovary produce substances necessary for the germination of pollen tubes in bluebell.

Germination of pollen grains

The initial step in germination is the absorption of water from the stigma. The pollen of barley, wheat, rye, and many grasses, is extremely sensitive to desiccation. However, it bursts at once if placed in water. Absorption of water is followed by protrusion of the inner wall of the grain (the intine) through one of the pores of the thick outer extine. The pollen tube continues to elongate and soon penetrates the intercellular tissue of the stigma, facilitated by the secretion of appropriate enzymes.

Viability of pollen

In angiosperms pollen may be capable of resting for considerable periods without losing its power of germination but viability varies enormously. In *Hibiscus trionum* it is as little as three days. It can be as much as seventy-two days in *Narcissus poeticus*. However, the pollen of barley must be transferred immediately to the stigmas if fertilization is to be successful. Date palms are at the other extreme. Frequently they are pollinated artificially and Arabs often put pollen aside from year to year in case the male flowers fail to develop in any season.

It is interesting to note that two characteristics of the spore-bearing plants are retained by these pollen grains – the resting stage and dispersal. The period of rest, however, is usually limited, so that pollination can take place only for a short period after the dehiscence of the anthers.

Protection of the pollen

Pollen can be easily damaged by water, so that in many flowers there are special devices to protect it from rain – for example the hood of monkshood or the way in which the flower hangs down in the harebell. An extreme case of protection of both stamens and stigmas is the banana flower, in which the huge purple bracts open out in succession as the rows of fruits set.

Quite another way in which pollen is kept dry is by the movements of the flower itself. This usually coincides with other physiological processes such as the production of nectar. Thus flowers pollinated by insects in the evening remain closed during the day, while many flowers such as daisy, crocus, and tulip, which are normally open during the day, close at night or when there is a rapid decrease of temperature. Many flowers close during rain and open in sun. In most flowering plants the movement of the parts follows a rhythm which is modified by external factors such as temperature and light intensity; it appears that atmospheric humidity plays no part in this.

8.19

Pollen grains, ovules, and seeds

Notes on teaching

Microscope preparations of pollen grains germinating on a stigma amplify the work of the previous section. Evening primrose (*Oenothera lamarckiana*) or common chickweed (*Stellaria media*) are a useful source of material from which preparations can easily be made (see below). Prepared slides of *Oenothera* stigmas with germinating pollen can be obtained from biological suppliers.

The actual process of fertilization is impossible to demonstrate, so at this point we must resort to the use of diagrams. Comparison should be made with the process in animals.

Description of the fertilization process in the plant has been reduced to its simplest dimensions and all reference to separate generative and tube nuclei is omitted.

The function of the pollen tube is a useful point for discussion. As a vehicle for the transference of pollen nucleus from pollen grain to ovule, it is comparable to the various structures in animals used for the carriage of sperms from testes to egg. It is not intended in this section to go beyond the newly formed seed resulting from fertilization. Its development and the formation of the fruit follows later.

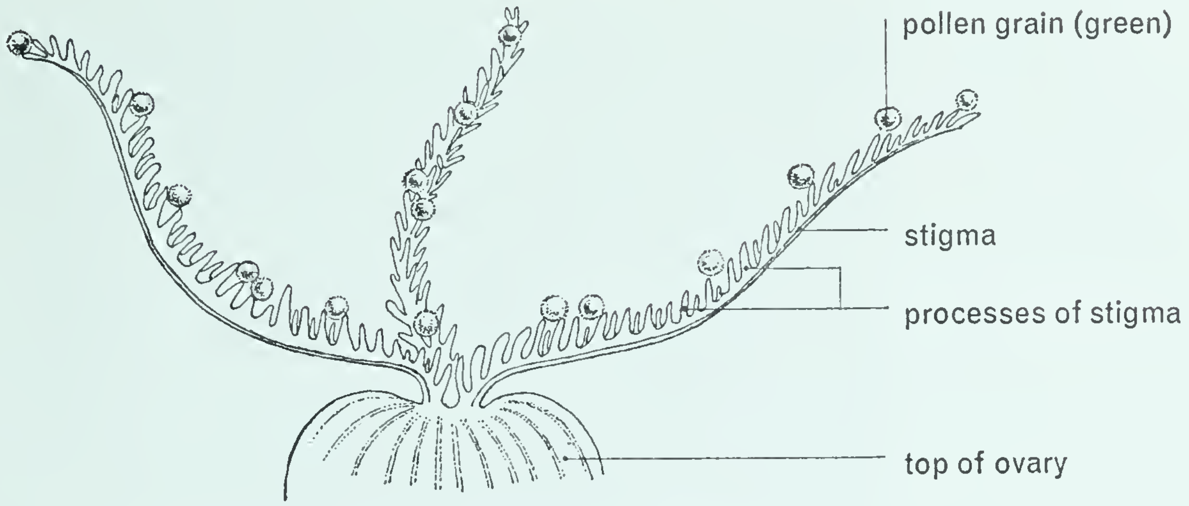
Making squash preparations

Oenothera lamarckiana

Pollinate the flowers and remove the styles after twelve to eighteen hours to demonstrate the growth of pollen tubes. Simultaneously fix and stain the styles in alcoholic iodine and potassium iodide (1 g iodine, 1·5 g potassium iodide, 100 cm^3 30 per cent alcohol), in which they can be examined or stored indefinitely. For examination squash the whole style between microscope slides. Use a binocular microscope.

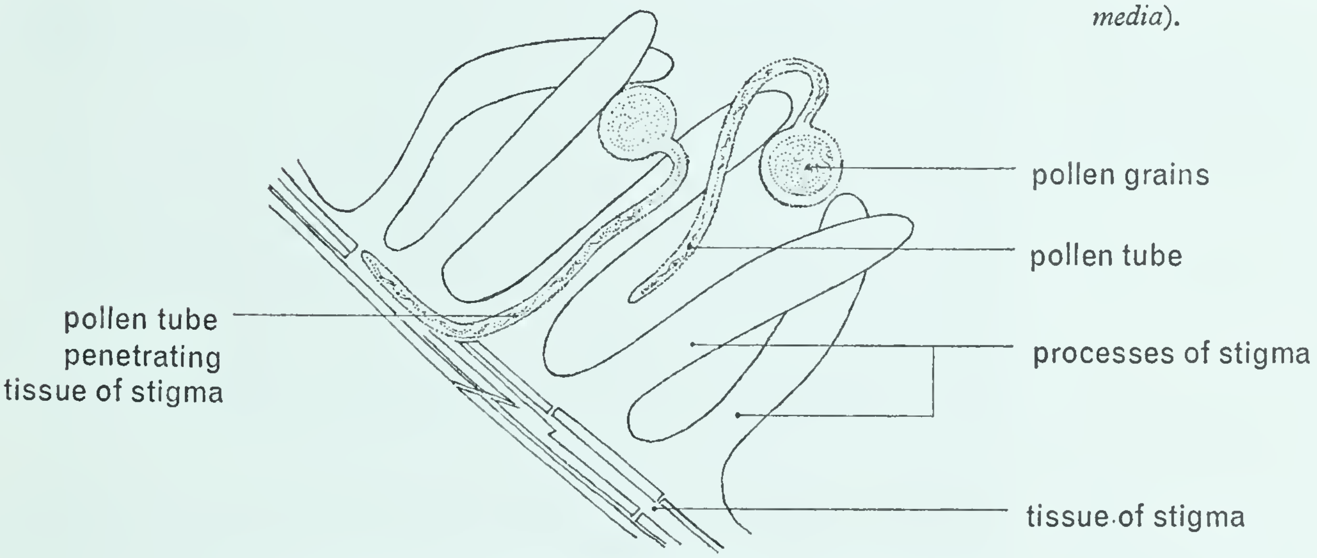
Stellaria media

Stellaria media is self-pollinated. The style is very short and the stigma trifid (see text figure 64*a*). The stigma together with a fragment of the top of the ovary is removed and stained lightly with methylene blue (the concentration is not important). It is then



a. Trifid stigma under low power

Figure G12.
Microscope preparation of pollen grains germinating on the stigma of common chickweed (*Stellaria media*).



b. Portion of stigma under high power

mounted on a slide under a cover-glass when slight pressure will squash the stigma. Pollen grains and pollen tubes stain blue, and show up well under high power against the processes of the stigma (see figure G 12*b*). Even without staining the pollen grains, which are green, and the colourless pollen tubes can be distinguished.

8.2
What happens if pollen is not allowed to reach the stigma?

Apparatus required per group of four or six
Two muslin bags
Growing radish plants in flower
Rubber bands or string
Garden canes
1 pair of sharp, pointed scissors
Hand lens × 8
Labels and coloured wool

Radish plants which have been allowed to grow until they flower are suitable for emasculation since they are open flowers and have only six stamens. They are normally cross-pollinated. Hence the chance is remote that a flower has already been pollinated while still in the bud stage. However, mustard or almost any kind of plant not normally self-pollinated would do equally well and it might be a good thing to try several different species. In the case of herbaceous or annual flowers, some kind of support will be required to prevent the muslin from breaking the flower stalk.

It is hard to tell whether the ovules have been fertilized or not when the swelling of the ovary does not become apparent within a few weeks. This should be borne in mind when selecting suitable material; the fall of petals does not necessarily indicate fertilization. Radish is particularly suitable because the undeveloped fruits persist and can be compared with the ripe ovaries.

It is important to leave the coloured wool attached to plant (B) so that it can be examined when the ripening of fruits is discussed in section 8.3.

8.21
Pollen from another flower and from the same flower?
Notes on teaching

The meaning of cross-pollination should now be quite clear. It remains to be shown that self-pollination is not only possible but quite often the usual state of affairs.

There are also a number of common garden flowers which are habitually self-pollinated, among them the garden pea. The sweet pea, so often described for its floral mechanisms designed to attract insects, in fact, is nearly always self-pollinated. Hence both the garden and sweet pea are unsuitable to use in the emasculation experiments because pollination takes place while the flowers are still in tight bud. Tulip is suitable for self-pollination experiments, although sweet pea or garden pea do equally well.

8.22
Some ways in which flowers ensure cross-pollination
Notes on teaching

White deadnettle has been used for the interest of its floral mechanism. Other flowers can, however, be substituted, particularly *Salvia*, sage, yellow deadnettle, and purple loosestrife. It is best to examine as many different ways in which cross-pollination can be brought about as time allows for.

Dichogamy as a means of ensuring cross-pollination

Dichogamy is the condition in which the male and female parts of a flower mature at different times. Two forms of dichogamy exist – *protogyny* in which the stigmas become receptive before the stamens are ripe and *protandry* in which the stamens ripen before the stigmas. Protandry is more common with many of the wind-pollinated monoecious trees (hazel) relying on protogyny. When so much pollen is broadcast, dichogamy is the only method of securing cross-pollination.

The interval between the receptiveness of the stigmas and the dehiscence of the stamens is usually about two or three days, but may be as long as nine days in the hazel. There seems to be no exact information available as to the length of time the stigmas remain receptive in the absence of cross-pollination, although this may be quite short.

Dichogamy is frequent in hermaphrodite flowers. Salvia, some of the willow herbs such as *Epilobium angustifolium*, and many of the *Compositae* and *Umbelliferae* are protandrous, while plantains and hellebores are protogynous.

In the grasses, protandry is usual, although many species are habitually self-pollinated before the flower opens. But the rapid growth of both the style and the stamen filaments renders the interval of time between shedding of pollen and opening of the stigmas extremely short. Often the stamens shed pollen just before the stigmas open, so that the stigmas are only exposed for a few minutes. In these circumstances self-pollination very often takes place.

8.3
The fruit ripens
Notes on teaching

The development of the seeds after fertilization and the growth of the ovary wall to accommodate them are the main points to stress here. It is important to establish that the seed is not a static entity but that it grows. Apple trees could be examined for fruits in different stages of development, and a ripe apple cut open to show the ripe seeds inside. The radish plant (B) could be re-examined to find out how far the fruits have developed. One of the fruits should be cut open to find the seeds.

If another kind of flower was selected for the experiments in section 8.2, the flowers which were not emasculated, should be examined. The greater the variety of fruits introduced, the better.

In contrast to large seeded plants, some producing large numbers of small seeds should be examined. Poppy or antirrhinum are good examples but there are also many suitable wild species such as campion, bluebell, shepherd's purse, and also other garden weeds.

8.31
Seeds are shed
Notes on teaching

If no large seeded plants which have formed ripe fruits are available, some in the process of ripening can be examined and contrasted with others such as campion and the others mentioned above which produce numerous seeds.

8.32
Sowing ripe seeds

Apparatus and materials required per group of four or six
3×4 inch plant pots (whale hide will do)
Damp soil
Trowel or spoon
6 pea seeds
10 lupin seeds (or nasturtium or sweet pea)
3 Polythene bags

Notes on teaching

The fact that plants grow from seeds is obvious, yet it is the logical ending to the sequence of events traced in this chapter – the production of pollen and ovules, germination of pollen grains, fertilization of ovule by pollen nucleus, development of seed and ovary wall, ripening of seed and fruit, shedding of seed, germination of seeds to give new plants.

By sowing now, seedlings should grow to a fair size by the end of the term. The class should be divided into groups of four or six.

Maize can be used as an alternative to pea in pot A. For pot C the class might collect their own ripe seeds for scatter-sowing. By enclosing each pot in a Polythene bag it should not be necessary to water them until the bags are removed. A convenient way of keeping the soil moist under dry classroom conditions is to stand the pots in a tray (or in a seed box with a sheet of Polythene underneath) in which there is a shallow layer of fine gravel or sand kept moist. A laundry sprinkler top fitted into the neck of a bottle is useful for top watering.

A good point for discussion is the question of whether large seeds always produce large plants and vice versa.

If the seeds are counted before being planted in pots A and B, the percentage germination can be calculated.

The pots containing the seedlings should not be destroyed when the work of this section is completed since they could well be used as material for alternative practical work when the growth of plants is considered in the next chapter.

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Further reading

*= reading for pupils

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Chapter 9

Growing up

Objectives

In this chapter the various problems of growth which have been considered in previous chapters are drawn together. Other methods of measuring growth in animals and plants are investigated and the pupils learn to make growth curves. From these they can extract data which could be used as a basis for the formulation of hypotheses about growth.

The order of development of the chapter is as follows:

1. Increase in size of an organism is indicated by its height (or length), girth, and surface area and is brought about by the division of body cells.

2. Environment affects growth.

3. Growth curves show that:

a. There is a relationship between the weight of food consumed and the rate of growth in immature mice to maturity, males and females growing at different rates.

b. The increase in the length of the tails of male and female mice is related to their increase in weight.

c. Most animals cease to grow at maturity. Growth in plants extends for a longer period.

d. Animals and plants grow according to a pattern.

4. The ages of some animals and plants can be calculated by examination of their structure.

5. The different parts of the body of an animal or plant do not grow at the same rate.

Suggestions for practical work

Class practical

		Page	
Section		*Text*	*Guide*
9.12	Finding out how mice grow up	146	102
9.13	Finding out about growth in plants	149	104
9.14	Making growth curves	149	104
9.15	Is there a relationship between the amount of food eaten and the rate of growth of mice?	152	105
9.21	Telling the age of trees		
	a. By examining the girdle scars on twigs	154	105
	b. By making an oblique cut across a twig	156	106

c. By counting annual rings on a tree stump	157	106

Planning practicals

Any study of the growth of an organism demands early preparation in order that the plant or animal will have reached the right stage of development for the start of the investigations.

The work of this chapter involves growth in mice as well as in wheat. Since the various experiments proceed simultaneously, in order to avoid confusion, careful records should be kept. It may be as well, therefore, to see that after initial explanation of the practicals, tables are prepared beforehand for the recording of results for each of the experiments requiring these.

Mice

Young mice at weaning (twenty days old) are needed for the start of the experimental work on growth. Thus the schedule outlined in the section below, entitled 'Rearing mice for growth experiments' should be consulted. From this schedule it will be seen that the mice should be mated about forty days before the start of the experiments.

Each week the mice are weighed and their tails measured. A good growth curve, starting to flatten out by week 8, should be obtained, but measurements could be continued until the end of term if this is possible.

Wheat

For section 9.13, pots or boxes of wheat seedlings are required. The shoots should be grown to a height of about 2 inches for the start of the work on increase in length. For this, soaked wheat grains should be sown about three weeks beforehand. Four-inch whalehide or plastic pots can be used. Prepare enough pots to allow one pot for each pair of pupils. Sow 15 grains in each.

9.1

What do you know about growth?

Notes on teaching

Throughout the introductory course there have been various investigations of growth so that before commencing the work of this chapter, which is largely practical, it is obvious that previous work on growth should be revised and suggestions obtained from the class as to what other aspects of growth could be considered, as well as other ways in which growth could be measured.

We are not concerned here with hormonal effects which will be dealt with later in the course.

9.11

Keeping mice for growth experiments

Cages

There are many types of cage on the market but in selecting the best one for school purposes, three factors have to be considered. These are (*a*) the size of cage to suit the number of mice to be kept, (*b*) whether the cages can be stacked, and (*c*) the ease of cleaning and the visibility of the mice to the class. To satisfy these requirements a plastic cage is probably the best choice. Text figures 80 and 81 show two types which are equally good. The Cambridge cage, designed by Dr M. E. Wallace, Cambridge University, Department of Genetics, consists of a plastic (polypropylene) bowl of internal dimensions: floor $10\frac{3}{4} \times 8\frac{3}{4}$ inch, height $3\frac{1}{4}$ inch. It is covered by a wire mesh lid sloping down towards the centre of the bowl.

On the lid a metal plate acts both as a cover to the nesting area and for holding food. A half or third-pint milk bottle supplies water. The cages can be obtained from Cope & Cope Ltd, Vastern House, 57 Vastern Road, Reading, Berks.

The Makrolon cage is of transparent plastic material of internal dimensions of 11×4 inches, height 6 inches. It is covered with a wire mesh lid sloping down at one end, the sloping part being divided by a partition into a section holding a water bottle, and one for food. Although the Makrolon cage has the obvious advantage of greater visibility, it has been found that some strains of mice do not produce such large litters in a transparent as in an opaque cage. The cages can be obtained from The North Kent Plastic Cages Ltd, Home Gardens, Dartford, Kent.

Nine or ten adults can be kept in either type of cage. For breeding, a trio of two does and a buck can be kept in one cage, and the does can nurse their litters at the same time without interfering with one another.

A battery of cages can be kept on racking made of metal strips fixed to the wall. A space of 4 inches between the top and bottom of vertically adjacent cages and one of 2–3 inches between cages side by side allows easy handling. Four vertical rows of three cages will occupy a wall area of about 5×2 ft and project about 10 inches.

A room temperature of around 20°C and reasonable ventilation with no draughts, provides good conditions for breeding. However, the design of the cages does allow reasonable results to be obtained in less than ideal circumstances.

Each doe should have her own record card which can be attached by a spring clip to the edge of the bowl of a cage. Each mouse can be marked (see 'Marking mice' in this section) and given a number and a record of each of her litters kept in columns. A description of the litter will give the number of each sex produced.

Doe No. 2 Parents – Doe No. 1. Buck No. 3

Date of mating	Stud buck	Date of birth of litter	Description of litter
3.5.66	no. 4	26.5.66	

Litter

Sawdust is frequently used as litter with a handful of dry hay, paper towelling, or shavings put in for bedding. It has been found, however, that a 1 inch layer of dry peat litter is the best since it absorbs all smell and the does will nest very readily straight in the peat. Peat is also highly absorbent, remaining dry in spite of urination.

Cleaning

Mice are very clean around the nesting area but urinate and defaecate during exercise and especially when eating and drinking. Once a week it may be necessary to change the peat litter although nesting does should be disturbed as little as possible.

Once a month the bowl should be washed in hot water (25°C) and if any infection occurs the bowl and the other parts of the cage should be washed out with a dilute solution of Jeyes Fluid and thoroughly rinsed.

Food and water

The mice obtain water from the capillary tubing inserted in the cork or cap of the water bottle and projecting about $\frac{1}{2}$ inch. A 1 inch length of capillary tubing 6–8 mm outer diameter and 1·25–2 mm inner diameter is used. The tubing in the bottle should be set at an angle of 30° from the horizontal and $1\frac{3}{4}$ inches from the cage floor. A bottle should be filled with water to $\frac{3}{4}$ inches below the inner edge of the cap, the cap or cork pressed on tightly and the bottle gently inserted into the cage. The water should be replenished when the inner projection of the capillary tube is out of water. The water bottle should be examined every day to see that the water can drip freely and that litter is not blocking the flow.

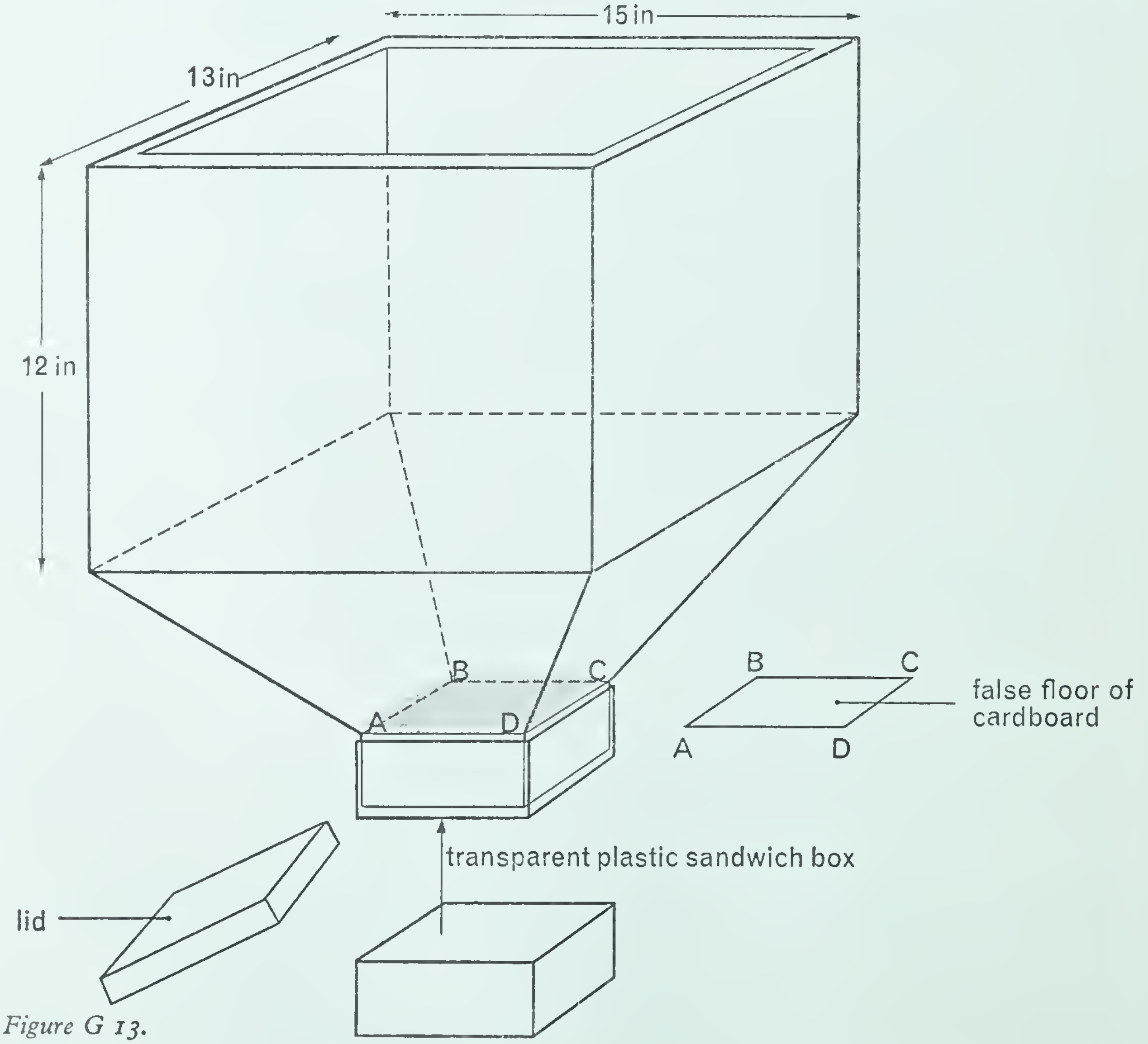

Figure G 13.
Cardboard funnel for transferring mice from the cage to the weighing box.

The most convenient form of food is in pellets. F.F.G. pellets, obtainable from E. Dixon & Sons, Crane Mead Mills, Ware, Herts, is specially hard containing 3–5 per cent molasses as well as the nutrients necessary for growth. It is therefore suitable for the experiments on the weight of food eaten and rejected since it does not crumble easily. The amount of food to be offered can be calculated from the figure of 60 g per day, being the average consumption of 10 adult mice. The food compartment of either of the cages recommended should hold sufficient food for a week.

Handling

The class will probably have to be taught how to handle the mice and this will best be done by lowering the whole cage into a funnel made from a cardboard box as illustrated in figure G13. It is then safe to lift the wire lid of the cage to extract a mouse without fear of others escaping while doing so. Instructions for the correct way of picking up a mouse and holding it for examination are given in the text. It is as well for each member of the class to have an opportunity of becoming used to handling. Apart from pregnant or lactating does and very young mice, the more handling a mouse gets the tamer it becomes.

Sexing

Sexing an adult mouse should present little difficulty since the genital papilla in the male is much bigger than the female and the testes have descended and are visible. The genital papilla in the male is about $\frac{1}{2}$ inch from the anus and in the female $\frac{1}{4}$ inch.

Immature mice are harder to sex but at 20 days this should not be difficult. Besides the distance of the genital papilla from the anus, two other criteria are used – in the male the skin between the genital papilla and the anus is furred while in the female it is naked, and in the female a slight depression behind the genital papilla marks the position of the imperforate vagina (see figure G14).

Breeding

Mice become sexually mature when they are six to eight weeks old. Gestation takes nineteen to twenty-one days and longer if the doe is suckling. The minimum generation interval is about nine weeks. The regular oestrous cycle in the mouse is four or five days in length. The doe therefore comes into heat every fourth or fifth night and will accept the male only at that time.

By keeping a trio of two does with one buck they should mate on the same day or within a day or so of each other. Mating can be detected by the presence of a vaginal plug formed by the male after copulation. It is a hard white substance which completely fills the vagina so that it can be seen with the naked eye. When it is more deeply placed, it can be detected with the aid of a small spatula, gently inserted into the vagina. For exact timing, a vaginal plug should be looked for every day. Although it is usually dispersed after twenty-four hours, it can persist for two or three days.

The young can be weaned at twenty days old.

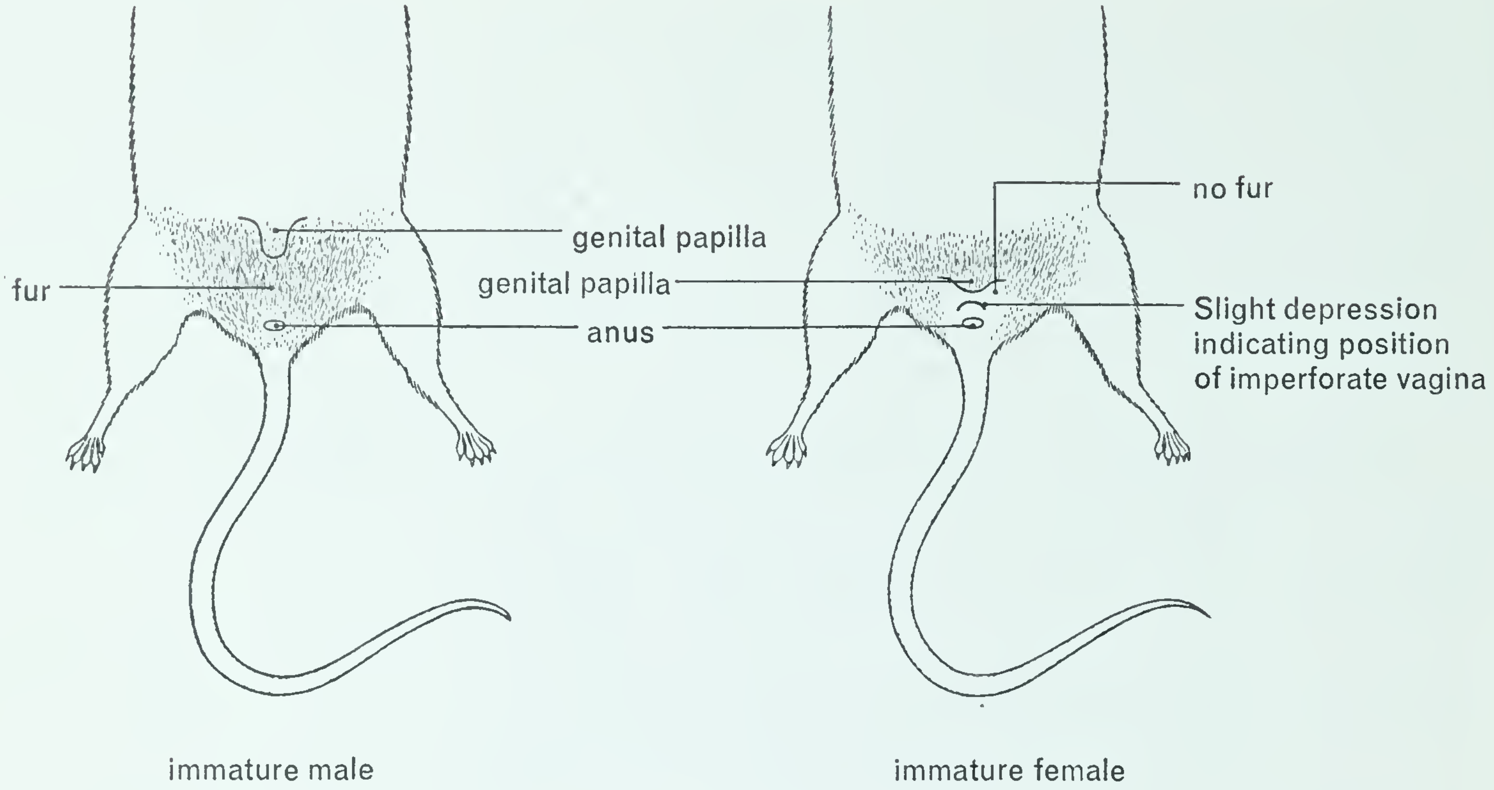

Figure G 14.
Sexing immature mice.

Marking mice

It is often necessary to mark mice and this is best done by using stains of different colours which dye the fur. These stains are not permanent and may have to be renewed from time to time.

Benzoyl alcohol	20 cm^3
Methyl alcohol	60 cm^3
Water	20 cm^3

Use the above mixture to dissolve and dilute the following dyes:

Basic fuchsin for red
Malachite green for green
Crystal violet for violet
A saturated solution of picric acid for yellow

Measuring the tails of mice

It may be difficult to hold a mouse still while measuring its tail; thus a special Perspex box, illustrated in text figure 84, has been devised. The diagrams in figure G15 show the dimensions of the box and the pieces of Perspex can be ordered already cut to size, for very little additional cost. The $\frac{1}{8}$ inch Perspex sheet (obtainable from Howard Goldstein Ltd, 161 High Street, Deritend, Birmingham 12, and many other suppliers of Perspex), is glued with Tensol No. 6 cement (obtainable from Imperial Chemical Industries Ltd, P.O. Box 52, 35 Queen Square, Bristol 1). Note that for best results, the gap between the two smallest pieces forming the 'V' into which the tail of the mouse fits, must be about $\frac{3}{16}$ inch at the bottom. Upwards centimetre scale tape called 'Scalafix' is obtainable from Fisons Scientific Apparatus Ltd, Loughborough, Leicestershire.

The mouse must be held so that its rump is tightly against the back of the box at the V.

Rearing mice for growth experiments

For these experiments it will be important to work with mice of the same age. Any breed of mouse, obtainable from pet stores, can be used, but preferably of a strain known to produce large litters. Several trios of mice should be kept, since at least five young mice of each six are required.

As outlined above (see 'Breeding') the two does should mate on the same day and produce litters on the same day. Since young males tend to eat more food and put on weight faster than the young females, separation of the sexes for the growth experiments is necessary and their growth rates can then be compared. Temperature also affects rate of growth, thus since the micro-temperature of the cage will vary according to the number of mice in it, it is important that the same number of males and females are used for the experiment if comparisons of growth rates are to be

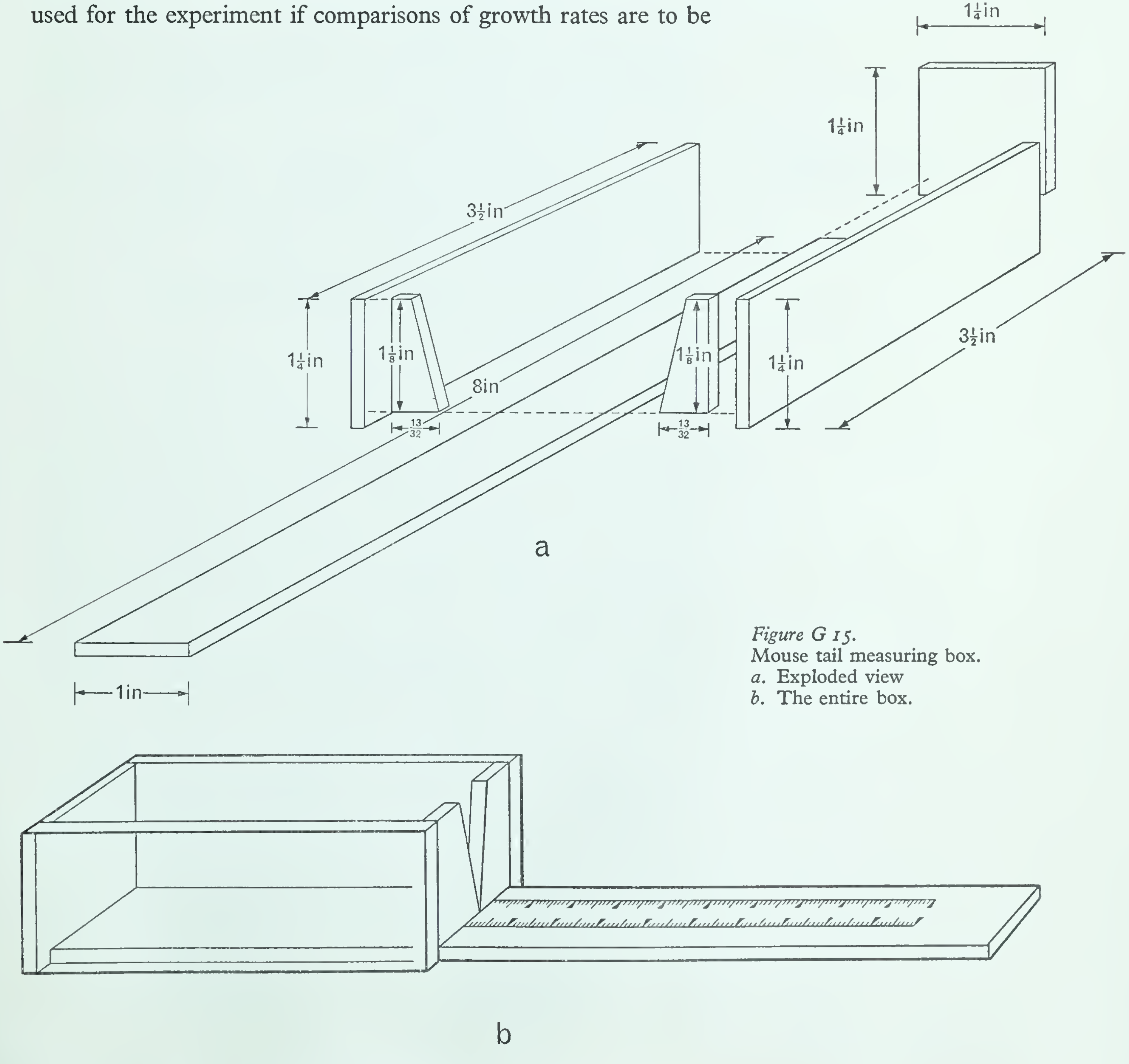

Figure G 15.
Mouse tail measuring box.
a. Exploded view
b. The entire box.

observed. Unless the mice are marked, individual weighings cannot be made. However, by averaging the totals a more accurate curve may be obtained.

A suggested schedule to be followed for the production of mice aged twenty days for the start of the growth experiments is:

Day-41	Place trio together in cage
Day-40	Mating takes place
Day-20	Birth of young
Day-0	Young mice 20 days old and ready for weaning and start of experiments

9.12

Finding out about how mice grow up

Notes on teaching

Apparatus required per class

4 mouse cages (preferably the Cambridge type) with water bottles, dry peat litter, and pellet food
At least 5 young female mice 20 days old
At least 5 young male mice 20 days old
} there should be the same number of each sex
1 balance (direct weighing)
1 cardboard box made into a funnel as described
1 square plastic box and lid for weighing the mice
2 mouse tail measuring boxes

In this experiment we are concerned with showing that length or height is not the only way in which growth can be measured although it is one of the indications that an animal is growing up. We also want to find out if animals increase in weight as they grow up and if there is any connection between increase in weight and increase in size of any other part of the body – length of tail for instance. Results of weighing are not likely to reflect a true picture of the average growth rate unless the mice are of fairly uniform size at the start of the experiment and unless at least five of each sex are weighed.

It is also important that the mice should be weighed at the same time of the day on each occasion since there is a considerable diurnal variation in weight.

Weighing

As a safeguard against loss of mice and consequent confusion in the classroom the funnel shown in figure G13 can be constructed from a cardboard box cut down to the dimensions shown and stuck together with Sellotape. A false floor of a loose square of cardboard is placed in the bottom of the funnel. A Perspex refrigerator box about 4×4 inches and $1\frac{1}{2}$ inches deep, with a loose fitting lid, is placed under the funnel to receive the mice. The whole cage of mice is then lowered into the funnel and the wire lid removed. The false floor is then removed and as the mice drop into the plastic weighing box, its lid is slipped into place.

A direct weighing balance is probably the easiest type to use, giving sufficient accuracy for this work. It is important that the young mice should not be chilled too much. Thus the plastic weighing box should be lined with absorbent paper wadding of known weight, but if this becomes damped with urine it should be removed after each weighing so as not to bias subsequent weighings.

The funnel can be used again for transferring mice, one at a time, to the mouse tail measuring box.

Too frequent handling tends to cause anxiety and consequent loss of weight, but this will depend upon the variety of mice used. For many breeds a twice-weekly weighing would be perfectly all right.

Amount of food

Five mice at forty-two days old will consume about 30 g of pellet food daily, so that the figure of 400 g given in stage 6 of the practical work (section 9.12) should be more than an adequate supply for a week for each cage during the growing period.

Alternative experiments using mice

1. Do all the mice of a single litter grow at the same rate?

For this experiment it will be necessary to mark the mice of one sex, when they are weaned at twenty days old, and to weigh them individually, keeping separate records of weight increase. They should be weighed in corked specimen tubes 4 × 1½ inches. A small hole is bored in the cork through which is placed a short length of glass tubing to admit air. By graphing the results recorded over a period of four weeks the different rates of growth will be apparent.

2. Growth rates of large and small families of pre-weaners

This experiment will show the effect of the amount of available food supplied by the mother on the rate of growth. Young mice of one litter are removed at two or three days old from the mother (leaving not less than two) and fostered with another mother with a litter of the same age, so that she has not more than ten young in all. The small litter and the large litter are then weighed separately, as a family, and records kept until weaning, of their average weight increase. By graphing results, two growth curves will be obtained, which can be compared.

3. Increase in weight of female mice during pregnancy

Two or three does which have recently been mated at the same time, are weighed separately during the period of gestation and their increase in weight recorded and graphed. Such records should illustrate that (*a*) an increase in weight is an indication of pregnancy, and (*b*) the increase in weight can be related to the number of young born. The does should be weighed again after parturition to show the sharp decrease in weight and consequently the proof of (*a*) above.

The pregnant does used to produce young for the growth records in 9.14 could be used for this alternative experiment.

9.13
Finding out about growth in plants
Notes on teaching

Apparatus required per pair

1×4 inches whalehide plant pot with wheat (barley or oat) seedlings grown to a height of about 2 inches

Rulers with centimeter scale

Since linear growth is to be measured, any of the grain corns will do – barley, oats, or wheat – and if the grains are soaked before sowing for twenty-four hours, germination is quicker. Sow 15 grains in boxes of John Innes potting compost or in a mixture of sandy soil to which Vermiculite is added. Start to measure the seedlings when they are about two inches high. Because of this, the grains should be sown about fourteen days or so beforehand and kept in a warm place. Sowing 15 grains will ensure that at least 10 will germinate. Surplus seedlings can be removed. A series of at least four measurements should be made at weekly intervals in order to obtain a good growth curve. Any difference in the height of the seedlings in a pot will emphasize individual variation and the consequent importance of averaging results.

9.14
Making growth curves
Notes on teaching

Average weight is plotted against age in days and to this graph a further curve is added (see section 9.15) to show the weight of food consumed. Since graphs are to be made using measurements in grams and centimetres, help will be required with the scales to be used for the vertical and the horizontal axes. It will be advisable to use a hypothetical example on the blackboard to assist with the compilation of the graphs to be constructed from individual records.

The questions are designed to assist with deductions made from the graphs and possible hypotheses to be drawn. It is to be hoped that these will elicit other questions and hypotheses. The curves obtained for the increase in length of the tails in the male and female mice should follow that for their increase in weight. Thus it can be deduced that tail length is an indication of weight.

Growth curves

The typical growth curve is sigmoid, for by plotting weight against time, the slope of the curve at first increases. An inflection is then reached and the curve gradually flattens out to the plateau of adult body weight.

By expressing the increase in weight as a percentage of the adult weight, and by adjusting the time-scale for each species, it is possible to fit the growth of a number of species to a single curve. Man, however, proves to be an exception since he has an abnormally long juvenile period of growth.

Growth curves plotted for increase in height of plants may prove to be quite different since the growing period for most plants continues throughout most of the life of the plant, although probably more rapid at first.

Thus there are opportunities for numerous measurements to be made on growth using different animals and plants and different parts of these organisms.

9.15
Is there a relationship between the amount of food eaten and the rate of growth of mice?
Notes on teaching

The average weight of the food consumed by the mice during the growing period should be added to the graphs of the increase in weight drawn in section 9.14.

The amount of food consumed by the males will probably be greater than that eaten by the females, illustrating the point that the rate of growth in both sexes, although different, can be related to the amount of food eaten.

The number of mice used will affect the growth rate not only because with a larger number there is more competition for the food but because a larger number increases the micro-environmental temperature of the cage and an increase in temperature increases growth rate up to the optimum temperature, in the case of mice, of about 20°C.

Alternative experiment to show the effect of exercise on growth

By using an exercise wheel in one cage and no wheel in another and by keeping the same number of males or females in each cage, the effect of exercise on growth can be demonstrated.

9.2
Telling the ages of animals
Notes on teaching

By now it should be evident that the graphing of growth curves can show a number of interesting things about growth and it may be as well to summarize these before proceeding to the important question of finding out from such curves the point at which growth ceases and the animal becomes adult.

It is important, however, to realize that in some animals growth proceeds throughout life. The guppy, a common fish often kept in aquaria, is an example.

Fish scales offer an opportunity for estimation of age by counting the rings. The scales from various Teleosts such as herring, haddock, or salmon might be tried.

The annuli or growth rings on fish scales correspond in many respects to the annual rings in the trunks of trees, both types of growth-ring being formed as the result of seasonally alternating periods of rapid growth when food is abundant, and very slight growth during the winter.

9.21
Telling the age of trees
Notes on teaching

Materials required per group of four or six

Twig of horse chestnut }
Twig of oak } Cut to the same length
Twig of ash, lime, or sycamore }
Penknife
Ruler

Horse chestnut probably provides the clearest examples of girdle scars and could be used to identify these in the first place. Thereafter a variety of twigs could be examined. For the practical work of this section, twigs, cut to the same length (quite long pieces should be provided) but from different trees, are provided so that not only is an estimation possible of the ages of those particular twigs, but also a comparison of their growth each year can be made. It will be found that an ash twig, for instance, grows very much more quickly than an oak twig. How does this relate to the growth of these trees as a whole and to the girth of the twig? By comparing the cut ends of ash and oak twigs of the same age and measuring the diameter of each, some idea of increase in girth as well as in length can be obtained.

By finding the stump of a freshly felled ash and oak it may be possible to count the annual rings in each and also to measure the diameter of the stump which would give an indication of the age and the rate of growth of each tree.

If tree stumps are not available, logs of various trees can be examined for annual rings. The distance between the rings in different logs can be measured and will indicate the relative increase in girth of the trees. The annual rings in oak will be much nearer together than those of a quick-growing tree such as sycamore, lime, or ash.

The rate of growth of a tree increases up to a certain age and then slows down. This is true of all trees but is very apparent in conifers. By examining the stumps of conifer saplings which have been removed in thinning operations in a plantation, it is not uncommon to find growth rings at least ½ inch wide indicating that the trunks had increased an inch or more in diameter every year. If such a rate of growth had been maintained, the trunks would have had a diameter of over 8 ft at the end of a hundred years. However this is not so. If the annual rings in the stump of an older conifer are measured, those towards the outside are found to be much closer together.

9.3
Patterns of growth
Notes on teaching

This section is concerned with two fundamental aspects of growth (*a*) that every organism has its own pattern of growth, and (*b*) that growth involves more than mere change of size for there is also a change in the proportions of the body.

All living things pass through three major phases of life – youth, maturity, and senescence, and in doing so they exhibit individual patterns of growth as well as changes in bodily proportions. The work already done in this chapter has shown that mice, wheat, and trees have patterns of growth and that man also follows a pattern of growth.

Upsetting the pattern of growth

Various environmental factors can upset the pattern of growth. Discounting the effect of hormones on growth, since these will be discussed in later years, other factors such as food supply, temperature, and, in the case of plants, light as well as other things can profoundly affect the pattern of growth. Two calves of the same age, one poorly fed and one well fed, will differ not only in size but in body proportions. The smaller beast will have more infantile proportions because the growth of later-developing parts are more severely affected by the limitation of food.

Pigs illustrate the effect of feeding on the development of different parts of the body. Young animals, well fed until they reach 200 lb, have shorter, lighter skeletons and much more fat than those which are poorly fed until they reach a body weight of 200 lb and which gain weight slowly during the growing period. These and other observations have been used in the production of meat, so that by selective feeding during the phases of development of the body structures, it is possible to produce more meat and less bone to the pound of body weight.

9.31

The span of life

Notes on teaching

Children are always interested in such questions as 'How long can an elephant live?' and perhaps the most important point to emphasize in discussing life spans is that although there are records for some exceptionally long-lived individuals, nevertheless the *average* life-span for that species may not be known. Records culled from zoos for some species may not give a true picture since, even disregarding the fact that in the wild state a species may not survive to old age, the artificial conditions of feeding and general care supplied by a zoo may greatly prolong the life of that species far beyond its normal span.

The first part of this chapter has been concerned with the period of growing up. This last section covers the whole life of animals and plants, including maturity and senescence.

Biological time

The length of life of an organism is normally calculated in months or years, yet it is clear that some animals and plants measure their life span in days, others in centuries.

All organisms live within the 'astronomical' time scale, which takes as its units external events, but there are other ways of experiencing time. The basis of a biological time scale lies in the fact that organisms compress within their life span the phases of growth, maturity, and senescence. There is no such thing as a common experience of time by all living things. Thus each according to its species, age, and environment has its own particular or biological time.

Du Noüy (1936) observes that in a man of sixty wound-healing takes place as if astronomical time passed five times as quickly as for a child of ten. The movements and reactions of an ageing individual appear to an observer to become slower while the person himself feels that the passage of external happenings is being accelerated, for time seems to pass more and more rapidly as an individual grows older.

Prolonging and shortening life

It is generally accepted that most living things live to six times the age represented by their maturation period. Thus by providing the appropriate conditions to retard growth artificially the duration of life can be extended considerably.

Woltereck (1961) gives an account of the work of McCay at Cornell University on longevity in rats which McCay divided into two groups. One group was fed normally while the other was given a diet containing sufficient vitamins and minerals but too few calories. The oldest rat on a normal diet reached an age of 965 days, whereas, after the same period, the undernourished rats were still not fully mature and were young and active. Thus by prolonging the period of maturation, the appearance of phenomena connected with old age was greatly postponed.

In cold-blooded organisms life span decreases with an increase of temperature. By raising the temperature 10°C the duration of life in *Drosophila*, for instance, is approximately halved. Indeed the duration of life for *Drosophila* is inversely proportional to the rate of energy expenditure. This equates with work done on humans, for it has been found that in feverish patients the duration of a minute at a temperature higher than normal seems longer than at normal body temperature.

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Chapter 10

Man and his environment

This final chapter is intended to be treated as background reading; it contains no experimental work. At the same time it is hoped that the ideas put forward will not only serve to draw together the aims and purpose underlying the whole of the introductory course, but that they will also provoke lively discussion and suggestions of various ways in which the children themselves can take part in the preservation of our environment. Conservation cannot be taught directly. In this course an understanding of the need for conservation will, it is hoped, arise from the development of the child's intuitional feeling for all living things. It is from this awareness of interrelationships that he will later be able to appreciate the clear and pressing arguments for conservation – in, for example, the prevention of soil erosion or the use of biological controls.

Much has been written about conservation and much has been achieved by the establishment of Nature Reserves and National Parks. By the same token a great deal still remains to be done in order to improve our own environment. On the one hand we establish parks and gardens in our cities, while on the other we permit unsightly, evil-smelling refuse tips on their outskirts. We ensure our own purified water supplies while allowing gross pollution of rivers, estuaries, and the seashore with sewage, oil, and chemical waste.

Revolted by the wholesale poisoning caused by some modern pesticides, we still speak hopefully of various methods of biological control. The spectacular achievements in controlling the Australian prickly pear or the success of ladybirds in the control of the scale pest of citrus fruits in California, are two notable examples. But it must be remembered that these successes were achieved, in both cases, against introduced pests which in their new environment got out of hand through the absence of their natural parasites, predators, and competitors.

Many attempts at biological control have achieved only limited success and in numerous instances where it has been employed the outcome is still far from certain.

If there are any lessons to be learnt by those now entering the world of the adolescent it is, perhaps, that growing up brings its responsibilities, not least in the preservation of our environment for our own sake and that of posterity.

Film Loop

A Film Loop entitled 'Predator/prey relationships' NBP–69, includes some excellent sequences of freshwater organisms – an aeschnid dragonfly nymph attacking and eating a young rudd, and in turn itself being eaten by the larva of the great diving beetle, *Dytiscus marginalis*. There is also a short sequence showing the pond snail, *Limnea stagnalis*, feeding on the remains of the rudd carcass left by the aeschnid nymph.

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Keys to small organisms in soil, litter, and water troughs

Film Loop notes